Effect of orthodontic treatment on the subgingival microbiota: A systematic review and meta-analysis

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Abstract: The aim of this systematic review was to assess qualitative changes induced by fixed appliance orthodontic treatment on the subgingival microbiota. Seven databases were searched up to August 2017 for randomized and nonrandomized clinical studies assessing the effect of orthodontic appliances on the subgingival bacteria in human patients. After elimination of duplicate studies, data extraction and risk of bias assessment according to the Cochrane guidelines, random-effects meta-analyses of relative risks (RR) and their 95% confidence intervals (CIs) were performed. According to controlled studies, the presence of Aggregatibacter actinomycetemcomitans in the subgingival crevicular fluid of orthodontic patients was increased 3-6 months after fixed appliance insertion compared to untreated patients (2 studies; RR = 15.54; 95% CI = 3.19-75.85). There was still increased subgingival prevalence of Aggregatibacter actinomycetemcomitans (3 studies; RR = 3.98; 95% CI = 1.23-12.89) and Tannerella forsythia in orthodontic patients up to 6 months after appliance removal compared to untreated patients. However, caution is warranted due to high risk of bias and imprecision. Insertion of orthodontic fixed appliances seems to be associated with a qualitative change of subgingival microbiota, which reverts to some extent back to normal in the first months after appliance removal. However, there is limited evidence on the timing and extent of these changes.

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Effect of orthodontic treatment on the subgingival microbiota: a systematic review and meta-analysis

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Structured Abstract

Objective: Aim of this systematic review was to assess the qualitative changes induced by fixed appliance orthodontic treatment on the subgingival microbiota.

Materials and Methods: Seven databases were searched up to August 2017 for randomized and non-randomized clinical studies assessing the effect of orthodontic appliances on the subgingival bacteria in human patients. After elimination of duplicate studies, data extraction, and risk of bias assessment according to the Cochrane guidelines, random effects meta-analyses of Relative Risks (RR) and their 95% confidence intervals (CIs) were performed.

Results: According to controlled studies, the presence of Aggregatibacter actinomycetemcomitans in the subgingival crevicular fluid of orthodontic patients increased 3-6 months after fixed appliance insertion compared to untreated patients (2 studies; RR=15.54; 95% CI=3.19-75.85). There was still increased subgingival prevalence of Aggregatibacter actinomycetemcomitans (3 studies; RR=3.98; 95% CI=1.23-12.89) and Tannerella forsythia in orthodontic patients up to 6 months after appliance removal compared to untreated patients. However, caution is warranted due to high risk of bias and imprecision.

Conclusions: Insertion of orthodontic fixed appliances seems to be associated with a qualitative change of subgingival microbiota, which reverts to some extent back to normal in the first months after appliance removal. However, there is limited evidence on the timing and extent of these changes.

KEYWORDS

orthodontics, orthodontic appliances, gingival crevicular fluid, clinical trials, meta-analysis

Manuscript

1 | BACKGROUND

1.1 | Rationale

Orthodontic treatment has been associated with certain adverse effects to the periodontium, which are generally thought to be transient and not related to any lasting tissue damage.[1,2] These include increased clinical plaque/bleeding indices,[3] enlargement of gingival pockets,[3] marginal bone loss,[3,4] and quantitative or qualitative changes in the oral microbiota.[5,6] Orthodontic treatement-induced microbial changes[7-10] can be attributed to the plaque-retentive characteristics of orthodontic appliances, whilst deepening of the gingival crevice through hyperplasia might offer a favorable environment for periodonto-pathogenic anaerobic bacteria.[11,12]

As far as intraoral microbial populations are concerned, considerable differences exist between the supragingival and subgingival microbiota in both health and disease.[13] Changes in the subgingival microbiota have been studied extensively (for a review see another source[14]) and a direct correlation has been found between microbial changes and the transition from periodontal health via gingivitis to periodontal disease.[15] These changes have been shown to be more pronounced in subgingival rather than supragingival plaque.[13] Additionally, members of the 'red' or 'orange complex' and specifically *Tannerella forsythia* (T.f.), *Porphyromonas gingivalis* (P.g.), *Aggregatibacter actinomycetemcomitans* (A.a.), and *Prevotella intermedia* (P.i.) have been found more frequently in patients with inflammation and periodontal pockets than in healthy subjects.[14] Therefore, it would be interesting to investigate the extent to which orthodontic appliances are associated with any qualitative changes in subgingival bacterial populations.

The only existing systematic review of treatment-induced subgingival changes[6] assessed a limited number of databases, whilst methodological issues, such as choice of a statistical model for data synthesis,[16,17] lack of subgroup analyses for potentially modifying factors,[18] and grading of clinical recommendations with the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach[19] justified this study.

1.1 | Objective

The aim of this systematic review was to assess evidence from clinical studies on human patients about qualitative changes in the subgingival microbiota induced by orthodontic treatment.

2 | MATERIALS AND METHODS

2.1 | Protocol, eligibility criteria, and registration

The protocol for this review was made *a priori* based on the PRISMA-P statement,[20] registered in PROSPERO (CRD42015029952), and all *post hoc* changes were appropriately noted. According to the PICOS schema, parallel or split-mouth randomized clinical trials and non-randomized cohort studies on human patients assessing the effect of any kind of orthodontic treatment on changes of the subgingival microbiota were included. Cross-sectional studies, non-clinical studies, and studies with partial appliances (appliance placed on single teeth) were excluded. This systematic review was conducted and reported according to Cochrane Handbook[21] and PRISMA statement,[22] respectively.

2.2 | Information sources and literature search

A total of seven electronic databases were searched systematically by one author (SNP) without any limitations from inception up to August 15th, 2017 (Appendix S1). Four additional sources (Scopus, Google Scholar, ClinicalTrials.gov, and ISRCTN registry) were manually searched for additional trials or protocols by the same author. Authors of included trials were contacted for additional missing or ongoing trials. No limitations concerning language, publication year or status were applied. The reference lists of the included trials and relevant reviews were manually searched as well.

2.3 | Study selection, data collection, and risk of bias in individual studies

Titles, abstracts, and full texts of studies identified from the literature search were screened by one author (SNP) with a subsequent duplicate independent checking against the eligibility criteria (Appendix S2) by another author (GMX), while conflicts were resolved by the last two authors (MTC, TE).

Characteristics of included trials and numerical data were extracted in duplicate by two authors (SNP, GMX) using pre-determined and piloted extraction forms. Piloting of the forms was performed during the protocol stage until over 90% agreement was reached.

The risk of bias of the included randomized trials was assessed using Cochrane's risk of bias tool,[21] while the risk of bias of the non-randomized studies was assessed with a checklist based on the Downs and Black tool and Cochrane Bias Methods Group tool (Appendix S3). Likewise, the risk of bias was assessed in duplicate by two authors (SNP, GMX) with the same way to resolve conflicts as above.

2.4 | Data synthesis

As the effect of orthodontic therapy on subgingival microbiota is bound to be affected by many factors, including type of orthodontic appliances, treatment duration, patient oral hygiene, and biological profile,[23-25] a random-effects model was deemed appropriate to incorporate this variability.[16] The novel Paule-Mandel random-effects estimator was used instead of the DerSimonian and Laird one, as it outperforms the latter.[17]

Relative Risks (RR) with their corresponding 95% Confidence Intervals (CI) were chosen as effect measures from binary outcomes of either controlled studies (with treated and untreated groups) or uncontrolled studies (only treated groups). The main scope of this review was to assess if orthodontic patients have increased prevalence of periopathogenic bacteria compared to untreated patients, based on controlled studies including treated and untreated patients. Secondarily, an explorative analysis was undertaken to assess the effect of insertion (or removal) of orthodontic appliances on the prevalence of periopathogenic bacteria within treated patients in a ‘before-and-after fashion’. As before and after data are correlated, standard errors of the effect sizes were appropriately adjusted (Appendix S4).

Absolute and relative between-trial heterogeneity was quantified with the tau^2 metric and the I^2 statistic, respectively. The latter is defined as the proportion of total variability in the results explained by heterogeneity, and not chance.[26] 95% Confidence Intervals (CIs) around all heterogeneity measures were calculated to quantify existing uncertainty. 95% predictive intervals (PrIs) were calculated for meta-analyses of three trials or more, to incorporate existing heterogeneity and provide a range of possible effects for a future clinical setting.[27] All analyses were run in Stata SE 14.0 (StataCorp, College Station, TX) by one author (SNP) and the dataset was openly provided to increase transparency.[28] A two-tailed P-value of 0.05 was considered significant for hypothesis-testing, except for a 0.10 used for the test of heterogeneity, due to low power.[26]

2.5 | Quality of evidence

The overall quality of controlled evidence (confidence in effect estimates) for each outcome was rated using the GRADE approach.[19] The minimal clinical important, large, and very large effects were defined a priori (Appendix S4) and used to augment the produced forest plots with contours denoting effect magnitude. The number needed to treat was used to clinically translate the results of statistically significant meta-analyses of binary outcomes.

2.6 | Additional analyses

Possible sources of heterogeneity were a priori planned to be sought through mixed-effects subgroup analyses and random-effects meta-regression for meta-analyses of at least fives studies (Appendix S4). Indications of reporting biases were planned to be assessed for meta-analyses of at least ten studies (Appendix S4).

Robustness of the results was planned a priori to be checked based on (i) inclusion of low risk of bias studies, (ii) inclusion of most precise studies, and (iii) improvement of GRADE.

3 | RESULTS

3.1 | Study selection

The literature search yielded a total of 193 hits as of August 2017 (Figure 1), 75 of which proceeded to full text assessment after eliminating duplicates and ineligible studies by title or abstract (Appendix S5). Finally, a total of 29 papers were identified as eligible for inclusion in the present systematic review. After pooling multiple publications relating to the same study, a total of 24 unique clinical studies were included.

3.2 | Study characteristics

The descriptive characteristics of the 24 included studies can be seen in Appendix S6. From these, 3 (13%) were randomized clinical trials, 5 (21%) were prospective non-randomized studies, and the remaining 16 (67%) were non-randomized studies of unclear design. The included studies were published between 1997-2016 and had been conducted in university clinics in 13 different countries. Overall, 980 patients were included with a mean age of 17.1 years (from the 19 studies reporting age) and with 291 (42%) patients being male (from the 22 studies reporting sex).

From the 24 included studies, all (100%) assessed the effect of fixed appliances (including one study and two studies using lingual and self-ligating appliances, respectively), one study (4%) additionally assessed the effect of removable appliances, and only 4 (17%) used an additional separate untreated control group for comparisons. The majority of studies (n=21; 88%) investigated changes in the subgingival microbiota after appliance insertion, while 8 studies (33%) investigated changes after appliance removal (and an overlap of 5 studies investigated both). The vast majority of studies assessed microbiological outcomes in a qualitative or quantitative manner using polymerase chain reaction and cell culture techniques.

3.3 | Risk of bias within studies

The risk of bias assessment for randomized and non-randomized studies is separately reported in summary in Figures 2a-2b and in detail in Appendix S7a-7b. All randomized trials were judged as having high risk of bias, due to issues in the random allocation of patients in groups and the lack of outcome assessor blinding. Likewise, all non-randomized studies presented high risk of bias for at least one domain, with the most problematic ones being lack of blinding, choice of statistical methods, and incomplete reporting. The latter was of particular interest, as it precluded in many instances the robust assessment of the studies' methods and conclusions.

3.4 | Results of individual studies and data synthesis

The complete list of all 41 different outcomes reported in the included studies can be seen in Appendix S8a-S8b. As all controlled studies reported on the subgingival detection of various bacteria in patients during fixed appliance orthodontic treatment and untreated patients, this was adopted for the main analyses, the GRADE assessment, and conclusions. From all outcomes reported, only in three instances were at least two studies included that could be pooled with meta-analysis: (i) the prevalence of A.a. after appliance insertion and (ii) the prevalence of A.a. and T.f. after appliance removal (Table 1; Figure 3). The results indicated that 3-6 months after appliance insertion, A.a. was detected subgingivally significantly more often among patients treated with fixed appliances than untreated controls (RR=15.54; 95% CI=3.19-75.85). This difference seemed to diminish 1-6 months after appliance removal, but still remained significantly increased compared to untreated patients (RR=3.98; 95% CI=1.23-12.89). The same was seen for an increased detection frequency of T.f. up to 3 monts after appliance removal in the sulcus of treated patients compared to untreated patients (RR=2.25; 95% CI=1.41-3.61).

Additionally, differences in the subgingival detection of specific bacteria before and after fixed appliance insertion (or before and after appliance removal) among only treated patients from uncontrolled studies was reported secondarily to assess the effect of treatment. As far as treatment-induced changes from uncontrolled studies are concerned (Table 2), most studies indicated a tendency for increased detection of periodontopathogens in the sulcus of treatment patients after appliance insertion (positive RRs). However, in many instances considerable heterogeneity among reported results was seen (I^2>70% for four out of eight cases), caution is warranted in the interpretation of these metrics, as only few studies were included and great uncertainty existed around the heterogeneity estimates (95% CIs for I^2 included zero). After appliance removal a significant reduction in the detection of A.a., P.g., and Treponema denticola (T.d.). was seen within the first 3-6 months, with generally low heterogeneity. However, meta-analyses from both controlled (Table 1) and uncontrolled studies (Table 2) included few studies, which together with the presence of heterogeneity resulted in very imprecise random-effects predictions (95% predictions included both favorable and unfavorable outcomes). Therefore, no consistent conclusions can be made on a universal basis.

3.5 | Risk of bias across studies

All three meta-analyses of controlled evidence were judged to be of very low quality according to GRADE (Table 3), due to the inclusion of potentially biased non-randomized studies and the limited sample, which could lead to imprecise estimates. Although the number needed to treat indicated considerable effect magnitude in two instances (prevalence of A.a. after fixed appliance insertion and prevalence of T.f. after appliance removal), GRADE was not upgraded due to existing problems with included studies.

3.6 | Additional analyses

No subgroup analyses could be performed for the review's main comparison using controlled evidence, as less than 5 studies were included. However, subgroup analyses/ meta-regressions were performed for meta-analyses of uncontrolled evidence (Appendix S9a-S9b). No significant influence on the subgingival response to orthodontic treatment was found for patient age, sex, type of brackets used (lingual or labial), level of subgingival measurements (patient- or tooth-level), and measured tooth (incisor or molar). Significant differences according to the sampling time-point were found for the detection of both P.g. and T.f. in the sulcus of treated patients after appliance insertion (Appendix S9a). In both instances this pattern could be characterized as an initial increase in the bacterium's presence during the first three months, which falters during the subsequent 3 months, and then manifests more intensively 6 months after appliance insertion. This could indicate that some months are needed until the initial stimulus of orthodontic appliances with their plaque-retentive role can lead to a change of the subgingival microenvironment. A similar observation was made for the effects of appliance removal (Appendix S9b). Likewise, a reduction tendency in the detection of P.g. was seen within the first 3 or 6 months after appliance removal, which further increased and became statistically significant after 6 months.

As only high risk studies were included and this justified the low GRADE given, no such sensititivy analyses could be performed with these factors. Sensitivity analyses including only the most precise 50% of studies of each meta-analysis led to consistent results (Appendix S10).

4 | DISCUSSION

4.1 | Summary of evidence

The present review summarizes clinical evidence up to August 2017 concerning the effects of orthodontic treatment with fixed appliances on the subgingival microbiota. Evidence from controlled studies indicates that appliance insertion is followed by a qualitative change in the composition of the subgingival bacterial population, with patients treated orthodontically being significantly more likely to present A.a. in the subgingival sulcus after appliance treatment compared to untreated patients. Additionally, the subgingival A.a. presence seemed to diminish in the first months after appliance removal, but was still significantly elevated compared to untreated patients. Finally, although the effect of appliance insertion on the T.f. levels could not be assessed through meta-analysis, orthodontic patients presented higher rates of subgingival T.f. detection up to 3 months after appliance removal. These changes can be justified by the notion that orthodontic treatment seems to be associated with increased intraoral microbial load that leads to periodontal inflammation.[3] It has been previously documented by comprehensive DNA-DNA checkerboard experiments that periodontal disease is associated with an increase in the subgingival proportion of "red" and "orange" complexes (from 25% to 35%) and a subsequent decrease of Actinomyces (from 47% to 38%).[13] It is important to note here that the contemporary view on the pathogenic potential of biofilms places the immunologic host response to a more central spot than oral ecology.[29] Although detection of periodontal pathogens in plaque samples among healthy patients prior to disease is not uncommon,[30,31] it has important implications in the prevention and treatment of periodontal infections.[13] It seems likely that periodontal pathogens colonize the supragingival plaque of periodontally healthy individuals for considerable periods of time prior to disease initiation. The development of gingival inflammation and the subsequent gingival enlargement might provide a habitat that fosters proliferation of these organsims, while the lateral periodontal pocket wall can provide attachment and nourishment.[13] It is therefore important to monitor the periodontal health of orthodontic patients and aim to keep the microbial load as low as possible before, during and after the course of orthodontic treatment.

The timing of these subgingival changes seems to be complex, but evidence from subgroup analyses of the present review (Appendix S9a-S9b) seems to indicate that periods of 6 months or longer might be needed in order to detect differences after either insertion or removal of the fixed appliances. The gingival enlargement that can be seen even among orthodontic patients with good oral hygiene and favors anaerobic bacteria might also contribute to the delayed microbial response, since although short-term signs of improvement are seen directly after appliance removal,[32] gingival enlargement can remain at least to some extent even several months after appliance removal.[33] Given the fact that many of the included studies reported on shorter follow-up periods, future research might focus on prolonged observation periods.

As far as modifying factors to the periodontal response are considered, no evidence for such a role could be found for patient-, site-, or appliance-related characteristics (Appendix S9a-S9b). An identified split-mouth randomized clinical reported that sites treated with self-ligating brackets presented lower microbial colonization and aspartate aminotransferase activity than sites treated with conventional ligation.[34] Another identified study reported that the percentage of P.g. in the total bacteria of patients treated with self-ligating appliances was lower than patients with conventional brackets.[35] As however, these studies were excluded from the meta-analyses of this review and a previous review reported overall non-signifcant differences in periodontal health,[36] caution is warranted in the interpretation of these two studies. Another identified split-mouth randomized trial compared the use of a self-etching adhesive containing an antibacterial monomer to conventional adhesive to bond orthodontic appliances, but did not find any significant differences in periodontopathogenic bacteria counts.[37] Finally, the single identified study that compared microbial changes between patients treated with fixed or removable appliances[38] reported that the latter influenced considerably less both supragingival and subgingival microbiota.

4.2 | Strenghts and limitations

The present study has some strengths, namely its protocol was registered a priori in PROSPERO[39] and it employed wide unrestricted literature changes and robust analytic methods (such as the iterative Paule–Mandel random-effects estimator that is less biased than the commonly used DerSimonian–Laird estimator).[17] Additionally, all post hoc deviations were listed in detail (Appendix S12) and the review's data set was transparently provided.[27]

However, the present study also has some limitations. First and foremost, there are a wide variety of outcomes (Appendix S8a-S8b) that were reported by single studies and could not therefore be pooled in meta-analyses. Importantly, the oral hygiene and plaque indeces of patients during treatment with fixed appliances was not consistently reported, as from the eight included studies (Table 2) only three reported the same plaque index and this precluded any meta-regressions. It must also be noted here that the presence or absence of specific bacteria is not necessarily disease-specific and similar bacterial species may be found in subgingival plaque samples taken from periodontally healthy and diseased subjects, although the proportions and levels of specific species can differ quite markedly.[30,40] Therefore, an identified study that reported the presence of high bacteria counts (instead of generally the detection of a bacterium) through orthodontic treatment[11] and was excluded from the meta-analysis was re-analyzed here (Appendix S11). It seems that although there is a statistically significant increase in the prevalence of high bacterial counts around 6 months after insertion for many pathogens (C.r., E.c., F.n., P.i., T.d., and T.f.), this does not continue throughout treatment or after appliance removal. One can therefore hypothesize that the subgingival changes during the first post-insertion months might be transient and more due to the extra efforts that the patient must invest to reduce the microbrial load on the newlyinserted orthodontic appliances. Another limitation of this review is the fact that the fulltexts of some potentially relevant studies could not be obtained to be assessed for inclusion. Additionally, the fact that only a subpart of all planned subgroup analyses and sensitivity analyses could be ultimately conducted (Appendix S12) means that the results' robustness might be compromised. Furthermore, mostly non-randomized trials were identified from the search and were included in the analyses, which could potentially influence the results.[41-43] Finally, as only patients from university clinics and only a handful of countries were included in the controlled trials, the generalizability of the review's conclusions might be limited to similar patients.

5 | CONCLUSIONS

The present systematic review of controlled clinical evidence indicates that orthodontic treatment with fixed appliances might be associated with an increased microbial colonization of the gingival crevicle in the first six months following the start of treatment. These changes seem to diminish slightly during the first six months after appliance removal, but still remained significantly increased compared to healthy patients. The current evidence base is however based on studies of potentially compromised internal validity, while additional studies are needed to precisely identify the time frame of microbial changes, as well as any potential patient- or appliance-specific factors.

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42. Papageorgiou SN, Kloukos D, Petridis H, Pandis N. Publication of statistically significant research findings in prosthodontics & implant dentistry in the context of other dental specialties. *J Dent.* 2015;43:1195-1202.
43. Papageorgiou SN, Xavier GM, Cobourne MT. Basic study design influences the results of orthodontic clinical investigations. *J Clin Epidemiol.* 2015;68:1512-22.

FIGURE LEGENDS

FIGURE 1 Flowdiagram for the identification and selection of studies in this systematic review. Tx – treatment

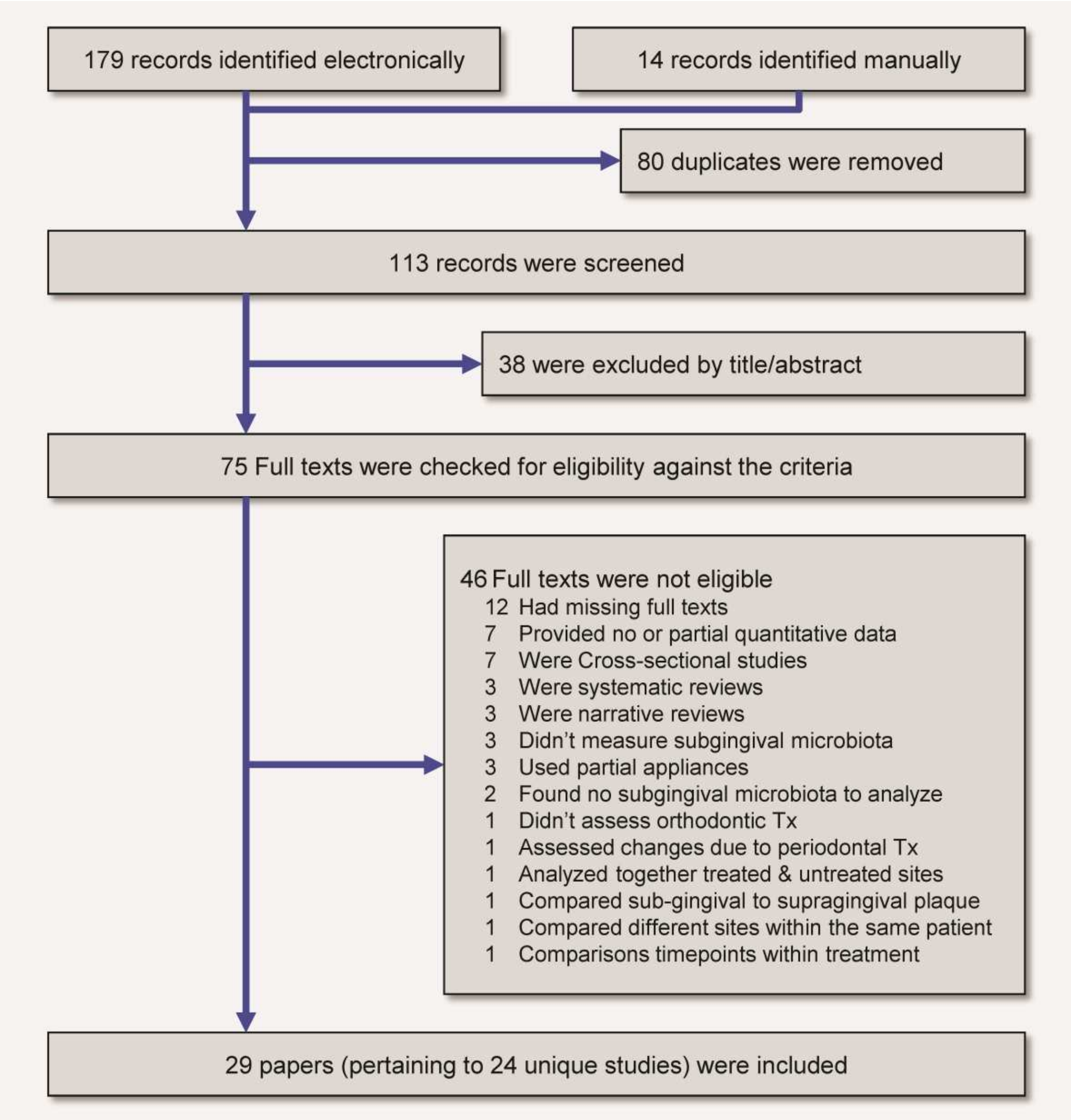

FIGURE 2a Summary of the risk of bias of included randomized trials

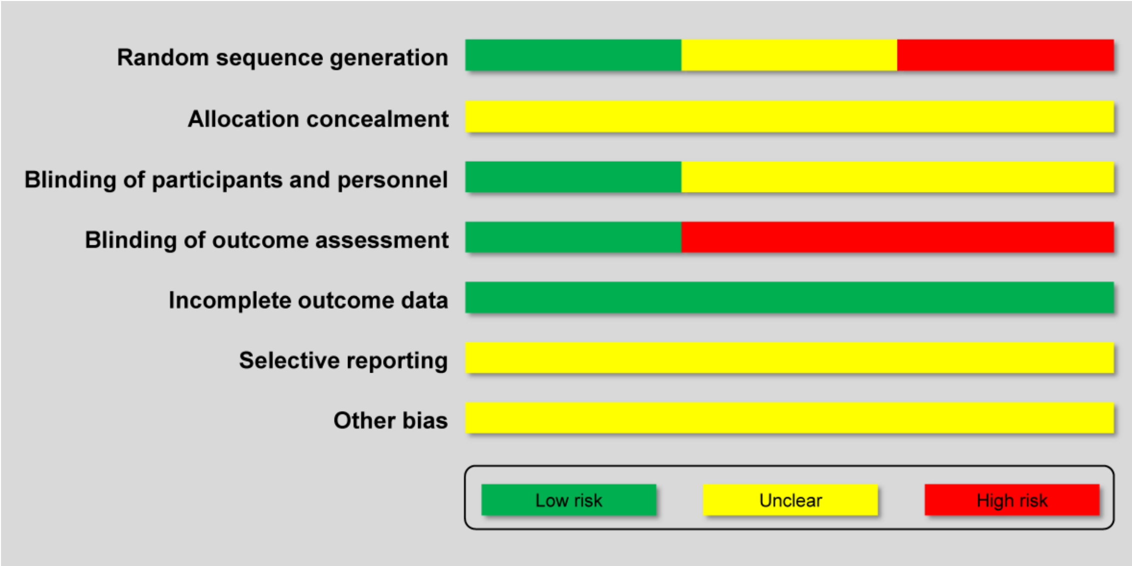

FIGURE 2b Summary of the risk of bias of included non-randomized studies

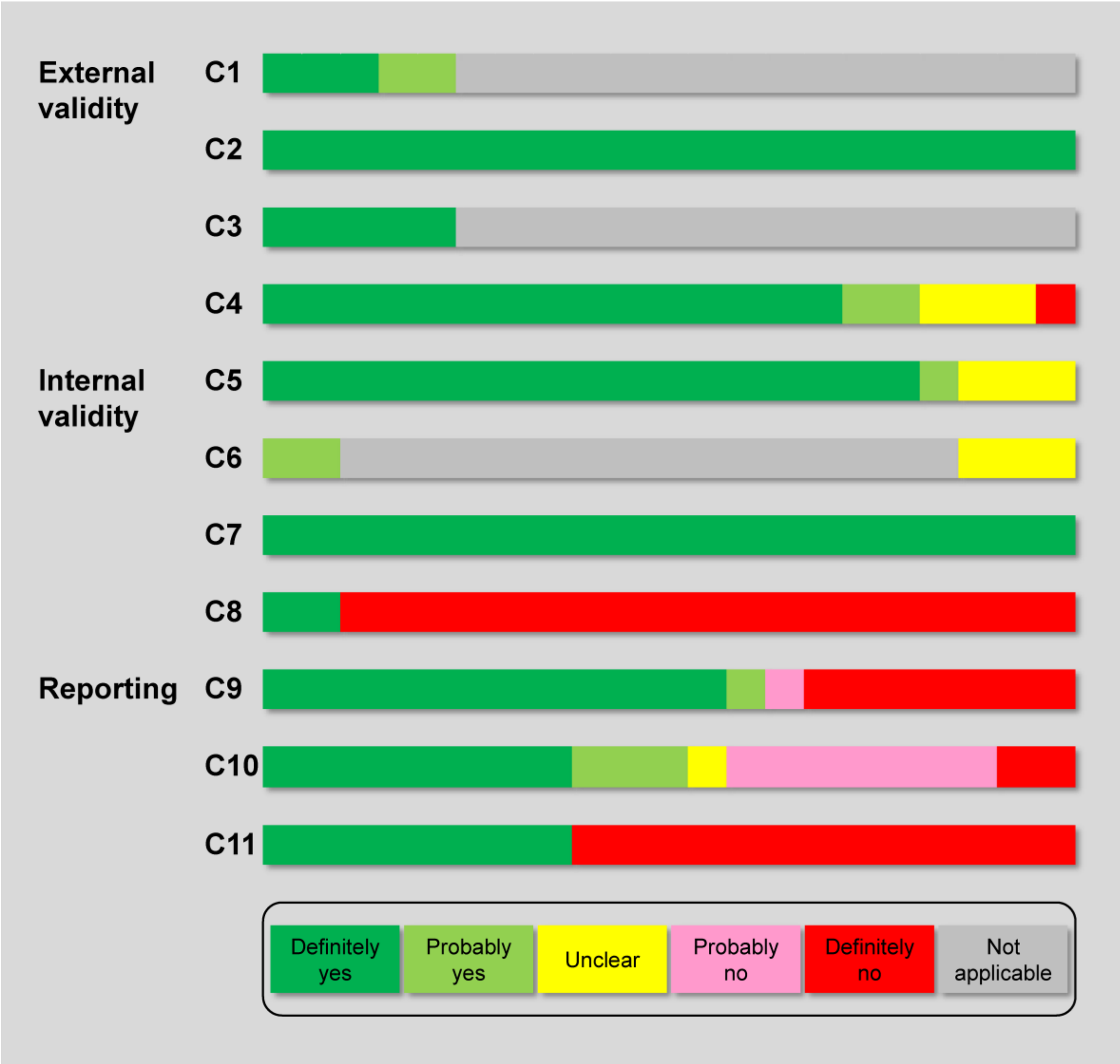

FIGURE 3 Contour-enhanced forest plot summarizing the meta-analyses from identified controlled clinical studies on orthodontic-related subgingival changes

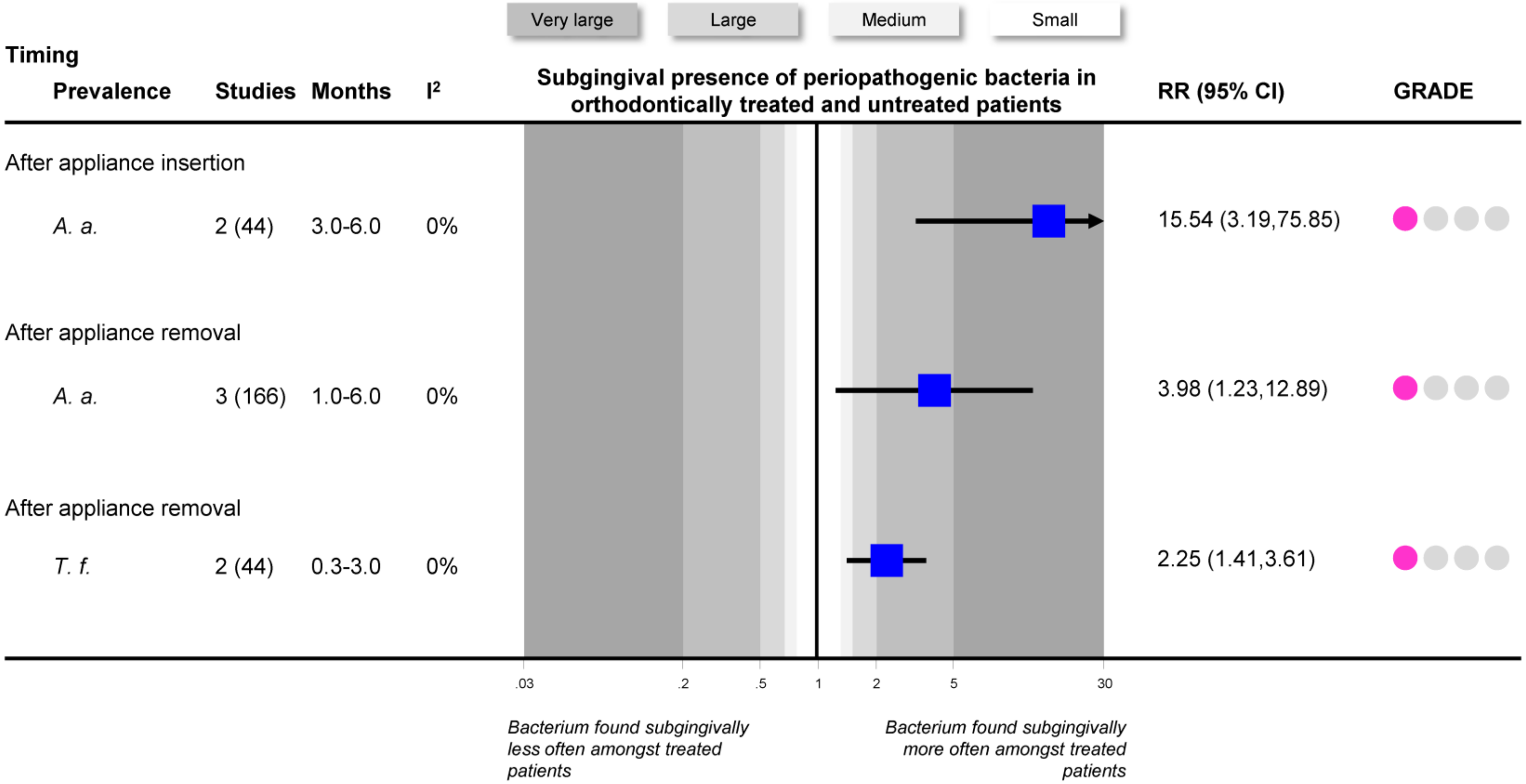

TABLE 1 Results of meta-analyses from identified controlled clinical studies (treatment and no-treatment groups) on orthodontic-related subgingival changes (referent: untreated control group; experimental: orthodontically treated group). Included in each meta-analysis are the time points with the longest follow-up reported in each study. Relative risks greater than 1 indicate that orthodontic patients have higher prevalence of each bacterium subgingivally than untreated (patients)

Comparison	Outcome	Studies (patients)	Months	RR (95% CI)	P		I^2 (95% CI)	tau^2 (95% CI)	95% prediction
Appliance insertion (referent: before insertion)									
	Prevalence of *A.a.*	2 (44)	3.0-6.0	15.54 (3.19-75.85)	0.001		0% (0-100%)	0 (0-324.34)	NA
Appliance removal (referent: before removal)									
	Prevalence of *A.a.*	3 (166)	1.0-6.0	3.98 (1.23-12.89)	0.02		0% (0-96%)	0 (0-24.77)	0-8083.08
	Prevalence of *T.f.*	2 (44)	0.3-3.0	2.25 (1.41-3.61)	0.001		0% (0-41%)	0 (0-0.08)	NA

RR, relative risk; CI, confidence interval; *A.a.*, Aggregatibacter actinomycetemcomitans; NA, not applicable; *T.f.*, Tanerrela forsythia.

TABLE 2 Results of meta-analyses from identified uncontrolled clinical studies (only treatment groups) on orthodontic-related subgingival changes. Referent: status prior to appliance insertion (upper part) or prior to appliance removal (lower part). Included in each meta-analysis are the time points with the longest follow-up reported in each study. Relative risks greater than 1 indicate that orthodontic patients have higher prevalence of each bacterium subgingivally after appliance insertion (or removal) than before

Comparison	Outcome	Studies	Months	RR (95% CI)		I^2 (95% CI)	tau^2 (95% CI)	95% prediction
Appliance insertion (referent: before insertion)								
	Prevalence of Aa	8	1.8-6.5	1.60 (0.58-4.41)		76% (0-96%)	1.31 (0-10.37)	0.07-34.51
	Prevalence of Cr	2	1.4-6.0	1.56 (1.24-1.95)		0% (0-98%)	0 (0-4.04)	NA
	Prevalence of Ec	3	1.4-6.0	0.99 (0.49-2.00)		86% (0-100%)	0.26 (0-40.01)	0-2789.26
	Prevalence of Fn	2	1.4-3.0	1.02 (0.77-1.35)		25% (0-100%)	0.02 (0-77.38)	NA
	Prevalence of Pg	8	1.4-6.5	0.82 (0.49,1.35)		47% (0-90%)	0.22 (0-2.18)	0.22-2.99
	Prevalence of Pi	5	1.4-6.0	1.23 (0.56-2.72)		73% (0-98%)	0.52 (0-10.62)	0.09-16.97
	Prevalence of Td	2	1.4-6.0	1.41 (0.86-2.32)		0% (0-100%)	0 (0-62.24)	NA
	Prevalence of Tf	6	1.4-6.0	1.83 (0.70,4.75)		74% (6-98%)	0.84 (0.02-12.78)	0.10-32.80
Appliance removal (referent: before removal)								
	Prevalence of Aa	4	0.3-1.0	0.33 (0.20-0.53)		0% (0-93%)	0 (0-4.61)	0.11-0.96
	Prevalence of Pg	6	0.3-6.0	0.69 (0.51-0.94)		15% (0-89%)	0.02 (0-1.03)	0.37-1.27
	Prevalence of Pi	4	0.3-3.0	0.95 (0.61-1.49)		0% (0-82%)	0 (0-1.00)	0.36-2.53
	Prevalence of Td	3	0.3-3.0	0.49 (0.36-0.65)		0% (0-97%)	0 (0-3.48)	0.07-3.24
	Prevalence of Tf	4	0.3-3.0	0.68 (0.51-0.92)		51% (0-98%)	0.05 (0-1.97)	0.22-2.10

Aa, Aggregatibacter actinomycetemcomitans; Cr, Campylobacter rectus; CI, confidence interval; Ec, Eikenella corrodens; Fn, Fusobacterium nucleatum; NA, not applicable; Pg, Porphyromonas gingivalis; Pi, Prevotella intermedia; RR, relative risk; Td, Treponema denticola; Tf, Tanerrela forsythia.

TABLE 3 GRADE Summary of Findings Table for meta-analyses of controlled clinical evidence on the effect of orthodontic treatment on subgingival microbiota

		Anticipated absolute effects[*] (95% CI)				
Outcome Studies (patients)	**RR (95% CI)**	**Control***	**Experimental**	**Difference**	**Quality of the evidence (GRADE)[a]**	**What happens**
Prevalence of Aa after appliance insertion 2 (44)	15.54 (3.19-75.85)	2.9%	45.1% (9.3% to 100.0%)	42.2% greater prevalence (6.4 to 97.1 greater) ***NNT of 3 (1 to 16)***	⊕⊖⊖⊖ very low[b,c] due to bias, imprecision	Orthodontic patients 3.0-6.0 months after appliance insertion have probably higher subgingival *A.a.* prevalence than untreated patients
Prevalence of Aa after appliance removal 3 (166)	3.98 (1.23-12.89)	2.0%	8.0% (2.5% to 25.8%)	6% greater prevalence (0.5 to 23.8 greater) ***NNT of 17 (4 to 218)***	⊕⊖⊖⊖ very low[b,c] due to bias, imprecision	Orthodontic patients 1.0-6.0 months after appliance removal might have higher subgingival *A.a.* prevalence than untreated patients
Prevalence of Tf after appliance removal 2 (44)	2.25 (1.41-3.61)	11.0%	24.8% (15.5% to 39.7%)	13.8% greater prevalence (4.5 to 28.7 greater) ***NNT of 8 (4 to 23)***	⊕⊖⊖⊖ very low[b,c] due to bias, imprecision	Orthodontic patients 0.3-3.0 months after appliance removal might have higher subgingival *T.f.* prevalence than untreated patients

Aa, Aggregatibacter actinomycetemcomitans; CI, Confidence interval; GRADE, Grading of Recommendations Assessment, Development and Evaluation; RR, relative risk; Tf, Tanerrela forsythia.
Qualitative changes in the subgingival microbiota of patients in receiving orthodontic treatment.
Patient or population: patients receiving treatment with fixed appliances for any kind of malocclusion.
Settings: university clinics (Brazil and Serbia).
[*] Reponse or risk in the control group is based on the average of the untreated control groups in included studies.
[a] Quality of evidence starts from low due to the inclusion of non-randomized studies.
[b] Downgraded by one due to risk of bias originating from methodological inadequacies.
[c] Downgraded by one due to imprecision originating from the inclusion of few studies with limited sample size.

Effect of orthodontic fixed appliance treatment on the subgingival microbiota: a systematic review and meta-analysis

Supplemental Material

Appendix S1. The electronic databases searched, the search strategy used, and the corresponding results (as of August 15th, 2017).

Appendix S2. List of inclusion and exclusion criteria.

Appendix S3. Guidance followed on using the risk of bias tool for non-randomized studies.

Appendix S4. Details on the review methodology.

Appendix S5. List of included and excluded studies, with the corresponding reasons.

Appendix 6a. Characteristics of the included trials (design, patient, and treatment characteristics).

Appendix S6b. Characteristics of the included trials (outcome details)/

Appendix S7a. Details of the risk of bias assessment for included randomized controlled trials.

Appendix S7b. Details of the risk of bias assessment for included non-randomized controlled trials.

Appendix S8a. List of all extracted outcomes from included studies: detection of single periodontopathogens in the subgingival sulcus.

Appendix S8b. List of all extracted outcomes from included studies: cumulative measurements in the subgingival sulcus.

Appendix S9a. Subgroup analyses and meta-regressions from uncontrolled studies of the effect of orthodontic appliance insertion on the subgingival microbiota of orthodontically treated patients.

Appendix S9b. Subgroup analyses and meta-regressions from uncontrolled studies of the effect of orthodontic appliance removal on the subgingival microbiota of orthodontically treated patients.

Appendix S10. Sensitivity analysis by including the most precise half of eligible studies (i.e. those with the smallest standard error).

Appendix S11. Results of the identified study Thornberg et al., 2009 that was excluded from the meta-analysis as it did not report overall detection of bacteria in the sulcus, but reported the presence of high bacterial counts in the sulcus. Results are presented as Relative Risks of each time point (from 6 to over 12 months after treatment and finally 6 months after appliance removal) compared to baseline (pre-insertion) status. Red boxed denote statistical significance at the 5% level.

Appendix S12. Supplementary Information on the review

Appendix S1. The electronic databases searched, the search strategy used, and the corresponding results (as of August 15th, 2017)

Database	Search Strategy	Limitations	Hits
MEDLINE searched through PubMed on August 15th, 2017 http://www.ncbi.nlm.nih.gov/pubmed/	orthodon* AND subgingiv* AND (microb* OR flora OR microflora OR environment OR sulc*)	Humans	68
Cochrane Database of Systematic Reviews searched on August 15th, 2017 http://onlinelibrary.wiley.com/cochranelibrary/search/	orthodon* AND subgingiv* AND (microb* OR flora OR microflora OR environment OR sulc*)	-	0
Cochrane Database of Abstracts of Reviews of Effects searched on August 15th, 2017 http://onlinelibrary.wiley.com/cochranelibrary/search/	orthodon* AND subgingiv* AND (microb* OR flora OR microflora OR environment OR sulc*)	-	0
Cochrane Central Register of Controlled Trials searched on August 15th, 2017 http://onlinelibrary.wiley.com/cochranelibrary/search/	orthodon* AND subgingiv* AND (microb* OR flora OR microflora OR environment OR sulc*)	-	15
Virtual Health Library searched on August 15th, 2017 http://regional.bvsalud.org/	orthodon* AND subgingiv* AND (microb* OR flora OR microflora OR environment OR sulc*)	-	7
Scopus searched on August 15th, 2017 http://www.scopus.com/	orthodon* AND subgingiv* AND (microb* OR flora OR microflora OR environment OR sulc*)	Dentistry	49
Web of Science searched on August 15th, 2017 https://isiknowledge.com/	orthodon* AND subgingiv* AND (microb* OR flora OR microflora OR environment OR sulc*)	Dentistry, oral surgery, medicine	40
Sum			-

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Appendix S2. List of inclusion and exclusion criteria.

Field	Inclusion	Exclusion
Patients	Patients of any age, sex, ethnicity, and malocclusion.	▪ Animal studies ▪ In vitro studies
Intervention (exposure)	Orthodontic treatment with any appliances (conventionally-ligated; self-ligated; lingual; buccal; metal; plastic; ceramic) with any kind of wire. The primary intervention will be full appliances (placed on all teeth – with the possible exception of 2nd and 3rd molars).	▪ Patient not receiving orthodontic treatment. ▪ Patients receiving partial appliances (brackets/bands placed on single teeth, single quadrants) for experimental reasons. ▪ Patients receiving or having received systemic antibiotic treatment less than a month before or during orthodontic treatment. ▪ Patients receiving or having received any kind of periodontal treatment less than a month before or during orthodontic treatment.
Comparison	A. ortho-Tx vs no-Tx ▪ No-Tx comprising data of the same patients before Tx ▪ No-Tx comprising data of other not treated patients B. ortho-Tx vs ortho Tx ▪ Effect of different adhesive materials/products ▪ Effect of different bracket materials ▪ Effect of different wire materials ▪ Effect of different ligature types (steel or elastomeric) ▪ Effect of different treatment modalities (self-ligating vs conventionally ligated; lingual vs buccal; fixed appliances vs removable appliances like functional appliances; expansion plates, etc; fixed-appliances vs aligners) C. no comparison ▪ For the descriptive analysis of subgingival microbiota in treated patients	
Outcome	Qualitative and quantitative analysis of the subgingival microbiota. All available time-points will be included and categorized accordingly into pre-treatment, short-term mid-treatment; long term mid-treatment; short-term post-treatment; long-term post-treatment.	▪ No clear mention / separate analysis of subgingival sampling. ▪ Clinical periodontal measurements. ▪ Supragingival microbiota measurements.
Study design	Randomized controlled trials or non-randomized, prospective or retrospective, cohort studies will be included.	

Tx, treatment.

Appendix S3. Guidance followed on using the risk of bias tool for non-randomized studies.

Nr	Category	Item	Guidance
1	External validity	Was selection of Tx and Ctr patients drawn from the same population and over the same period?	Patients from the Tx/Ctr patients are recruited from the same clinic at the same time (i.e. no other study sample is used as Ctr).
2		Can we be confident that Tx patients were treated?	Almost always yes if fixed appliances are used; for removable, look for any comment on compliance.
3		Can we be confident that Ctr patients were not treated?	Almost always yes as patients can clearly say if ortho Tx has taken place.
4		Can we be confident that periodontal health was not used as a patient selection criterion?	See for comment on the eligibility criteria/study selection that all patients were periodontally healthy or had no gingivitis/periodontitis prior to Tx Prior to ortho Tx all patients must be periodontally healthy (lege artis). However, patient selection during treatment cannot be based on periodontal disease (some studies select only treated patients with gingivitis/periodontitis for the study).
5	Internal validity	Are the characteristics of the patients included in the study clearly described ?	See if patient age and sex is reported on the table.
6		Did the study match Tx and Ctr patients for all variables that are associated with periodontal health (age, gender, smoking, diabetes) or did the statistical analysis adjust for these prognostic variables?	See if Tx/Ctr patients have similar age and gender distributions. If they have different characteristics, but these are taken into account in the stats (for example as covariates in ANCOVA) its ok.
7		Can we be confident in the assessment of periodontal disease (outcome measures valid and reliable)?	Almost always yes, as subgingival sampling and analysis is relatively straightforward and very specific.
8		Was outcome measurement performed blinded?	Search for "blind" or "mask" in the text.
9	Reporting	Is reporting of results complete and transparent (for continuous outcomes: n, mean, SD; for binary outcomes: sample and events; for calculated effect sizes: point estimate and SE or 95% CI)	FOR ALL SUBGINGIVAL OUTCOMES: n+m+SD for continuous / sample and event or % for binary outcomes; if any one outcome is not given quantitatively, rate negatively.
10		Were the statistical tests used to assess the main outcomes appropriate (normality/pairness)?	Normality need can be played down a little, as it might be assumed that the authors have checked this basic assumption. If however, non-parametric tests are used, the authors must report why. If clustered data are analyzed (for example more than 1 tooth measured per patient) then the authors need to take this into account and use appropriate tests. If they pool all measures sites within a patient and use simple t-tests or chi-square tests, rate negatively.
11		Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	Somewhat absurd, but let's stick to this item of the Newcastle Ottawa scale; p-values need to be given quantitatively and not as S/NS/<0.05, etc (<0.001 is ok however).

Tx, treatment; Ctr, control; SD, standard deviation; SE, standard error; CI, confidence interval.

*Appendix S4***.** Details on the review methodology.

Reporting bias (including publication bias)

Indications of reporting bias (including small-study effects and publication bias) were planned to be assessed with Egger's linear regression[1] test and contour-enhanced funnel plots[2] for meta-analyses of at least ten studies[3].

Data synthesis

Before-and-after data from included uncontrolled studies of only treated patients before and after appliance placement (or appliance removal) were handled appropriately to 'correct' for clustering using the design effect (according to method 2 described elsewhere[4]). The design effect was reverse-calculated from the identified study of Kim et al.[5] that provided tabulated data and P values from McNemar's tests. Initially, effect sizes (log relative risks and standard errors) were calculated ignoring clustering and then the square root of the design effect was used to multiply the standard errors with.

Initially planned subgroup analyses included subsets according to: (a) patient characteristics: age, sex, ethnicity, jaw, malocclusion type, patient compliance with the treatment or hygiene procedures; (b) treatment characteristics: type of appliances, material of appliances, ligation type of appliances, treatment duration; and (c) outcome measurement procedures.

GRADE approach

The minimal clinical important, large, and very large effects for continuous outcomes were conventionally defined in the review protocol as half[6], one, and two standard deviations, respectively (using the average standard deviation from the identified studies). The minimal clinical important, large, and very large effects for binary outcomes were conventionally defined in the review protocol as RRs greater than 1.30, 1.50, 2.0, or 5.0 (or their reciprocals)[7]. Finally, the optimal information size (i.e. required meta-analysis sample size) was calculated for each outcome independently for α = 5% and β = 20%.

References to Appendix S4

1. Egger, M. Davey Smith, G. Schneider, M. & Minder, C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* **315**:629–634 (1997).

2. Peters, J.L., Sutton, A.J., Jones, D.R., Abrams, K.R. & Rushton, L. Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *J. Clin. Epidemiol.* **61**:991–996 (2008).

3. Sterne, J.A. *et al*. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomized controlled trials. *BMJ* **343**:d4002 (2011).

4. Littell, J.H., Corcoran, J. & Pillai, V. eds. *Systematic Reviews and Meta-analysis*. Oxford: Oxford University Press, 92 (2008).

5. Kim, S.H. *et al*. Microbiologic changes in subgingival plaque before and during the early period of orthodontic treatment. *Angle Orthod.* **82**:254–260 (2012).

6. Norman, G.R., Sloan, J.A. & Wyrwich, K.W. The truly remarkable universality of half a standard deviation: confirmation through another look. *Expert Rev. Pharmacoecon. Outcomes Res.* **4**:581–585 (2004).

7. Schünemann, H., Brozek, J. & Oxman, A. eds. GRADE handbook for grading quality of evidence and strength of recommendation. Version 3.2. The GRADE Working Group; [updated March 2009] http://www.who.int/hiv/topics/mtct/grade_handbook.pdf (2009).

Appendix S5. List of included and excluded studies, with the corresponding reasons.

Nr.	Paper	Decision
Screening by title / abstracts		
1	Aass AM, Rossow I, Preus HR, Gjermo P. Incidence of early periodontitis in a group of young individuals during 8 years: associations with selected potential predictors. Journal of periodontology. 1994;65(9):814-9. Epub 1994/09/01.	Excluded by title
2	Alaluusua S, Kivitie-Kallio S, Wolf J, Haavio ML, Asikainen S, Pirinen S. Periodontal findings in Cohen syndrome with chronic neutropenia. Journal of periodontology. 1997;68(5):473-8. Epub 1997/05/01.	Excluded by title
3	Amiri-Jezeh M, Rateitschak E, Weiger R, Walter C. [The impact of the margin of restorations on periodontal health--a review]. Schweizer Monatsschrift fur Zahnmedizin 2006;116(6):606-13. Der Einfluss von Restaurations-randern auf die parodontale gesundheit--eine ubersicht.	Excluded by title
4	Baehni PC, Guggenheim B. Potential of diagnostic microbiology for treatment and prognosis of dental caries and periodontal diseases. Critical Reviews in Oral Biology & Medicine. 1996;7(3):259-77.	Excluded by title
5	Bate AL, Lerda F. Multidisciplinary approach to the treatment of an oblique crown-root fracture. Dental traumatology 2010;26(1):98-104. Epub 2010/01/22.	Excluded by title
6	Bueno L. Ortodoncia y periodoncia: dos especialidades que van de la mano [Orthodontics and periodontics: two specialties that go well together]. Rev Fundac Juan Jose Carraro.9(18):41-5.	Excluded by title
7	Canullo L, Quaranta A, Teles RP. The microbiota associated with implants restored with platform switching: a preliminary report. Journal of periodontology. 2010;81(3):403-11. Epub 2010/03/03.	Excluded by title
8	Caruso U, Nastri L, Piccolomini R, d'Ercole S, Mazza C, Guida L. Use of diode laser 980 nm as adjunctive therapy in the treatment of chronic periodontitis. A randomized controlled clinical trial. The new microbiologica. 2008;31(4):513-8. Epub 2009/01/07.	Excluded by title
9	D'Ercole S, Piccolomini R, Capaldo G, Catamo G, Perinetti G, Guida L. Effectiveness of ultrasonic instruments in the therapy of severe periodontitis: a comparative clinical-microbiological assessment with curettes. The new microbiologica. 2006;29(2):101-10. Epub 2006/07/18.	Excluded by title
10	Folio J, Rams TE, Keyes PH. Orthodontic therapy in patients with juvenile periodontitis: clinical and microbiologic effects. American journal of orthodontics. 1985;87(5):421-31. Epub 1985/05/01.	Excluded by title
11	Goelz L, Reichert C, Dirk C, Jaeger A. Retrospective investigation of gingival invaginations Part II: microbiological findings and genetic risk profile. Journal of Orofacial Orthopedics-Fortschritte Der Kieferorthopadie. 2012;73(5):387-96.	Excluded by title
12	Ho HP, Niederman R. Effectiveness of the Sonicare sonic toothbrush on reduction of plaque, gingivitis, probing pocket depth and subgingival bacteria in adolescent orthodontic patients. The Journal of clinical dentistry. 1997;8(1 Spec No):15-9.	Excluded by title
13	Johnson V, Johnson BD, Sims TJ, Whitney CW, Moncla BJ, Engel LD, et al. Effects of treatment on antibody titer to Porphyromonas gingivalis in gingival crevicular fluid of patients with rapidly progressive periodontitis. Journal of periodontology. 1993;64(6):559-65. Epub 1993/06/01.	Excluded by title
14	Lux CJ, Kugel B, Komposch G, Pohl S, Eickholz P. Orthodontic treatment in a patient with Papillon-Lefèvre Syndrome. Journal of periodontology. 2005;76(4):642-50.	Excluded by title
15	Mateu ME, Folco AA, Brusca MI, Benítez Rogé S, Calabrese D, Iglesias M, et al. Importancia de la terapia básica pre-tratamiento ortodóncico [The importance of pre-orthodontic treatment basic therapy]. Rev Fac Odontol (BAires).26(61):17-22.	Excluded by title
16	Nelson-Filho P, Carpio-Horta KO, Damiao Andrucioli MC, Feres M, Bezerra da Silva RA, Garcia Paula-Silva FW, et al. Molecular detection of Aggregatibacter actinomycetemcomitans on metallic brackets by the checkerboard DNA-DNA hybridization technique. American Journal of Orthodontics and Dentofacial Orthopedics. 2012;142(4):481-6.	Excluded by title
17	Özdemir B, Uraz A, Işcan D, Bozkurt S, Tuncer BB, Engin D, et al. Influence of Cervitec gel on periodontal health of patients wearing fixed orthodontic appliances. Journal of Dental Sciences. 2013.	Excluded by title
18	Peña Ruiz T, Martínez Brito I, Delgado Ramos A. Tratamiento integrado ortoperiodontal en la periodontitis juvenil: Presentación de un caso report. Revista Cubana de Estomatologia. 2007;44(4):0-.	Excluded by title
19	Petersilka GJ. Subgingival air-polishing in the treatment of periodontal biofilm infections. Periodontology 2000. 2011;55(1):124-42. Epub 2010/12/08.	Excluded by title
20	Pradeep AR, Karvekar S, Nagpal K, Patnaik K, Guruprasad CN, Kumaraswamy KM. Efficacy of locally delivered 1.2% rosuvastatin gel to non-surgical treatment of patients with chronic periodontitis: a randomized, placebo-controlled clinical trial. J Periodontol. 2015;86(6):738-45.	Excluded by title
21	Proff P, Reicheneder C, Faltermeier A, Kubein-Meesenburg D, Roemer P. Effects of mechanical and bacterial stressors on cytokine and growth-factor expression in periodontal ligament cells. Journal of Orofacial Orthopedics-Fortschritte Der Kieferorthopadie. 2014;75(3):191-202.	Excluded by title
22	Quirynen M, Dewinter G, Avontroodt P, Heidbuchel K, Verdonck A, Carels C. A split-mouth study on periodontal and microbial parameters in children with complete unilateral cleft lip and palate. Journal of clinical periodontology. 2003;30(1):49-56. Epub 2003/04/19.	Excluded by title
23	Ruiz TP, Brito IM, Ramos AD. Orthoperiodontal integrated treatment in juvenile periodontitis. A case report. Revista Cubana de Estomatologia. 2007;44(4).	Excluded by title
24	Schou S, Holmstrup P, Kornman KS. Non-human primates used in studies of periodontal disease pathogenesis: a review of the literature. Journal of periodontology. 1993;64(6):497-508.	Excluded by title
25	Tucker LM, Melker DJ, Chasolen HM. Combining perio-restorative protocols to maximize function. General Dentistry. 2012;60(4):280-7.	Excluded by title
26	Waerhaug J. Eruption of teeth into crowded position, loss of attachment, and downgrowth of subgingival plaque. American journal of orthodontics. 1980;78(4):453-9. Epub 1980/10/01.	Excluded by title
27	Wang L, Xie X, Weir MD, Fouad AF, Zhao L, Xu HH. Effect of bioactive dental adhesive on periodontal and endodontic pathogens. J Mater Sci Mater Med. 2016;27(11):168.	Excluded by title
28	Wheeler TT, McArthur WP, Magnusson I, Marks RG, Smith J, Sarrett DC, et al. Modeling the relationship between clinical, microbiologic, and immunologic parameters and alveolar bone levels in an elderly population. Journal of periodontology. 1994;65(1):68-78. Epub 1994/01/01.	Excluded by title

29	Zheng H, Xu L, Wang Z, Li L, Zhang J, Zhang Q, et al. Subgingival microbiome in patients with healthy and ailing dental implants. Sci Rep. 2015;5:10948.	Excluded by title
30	[No authors listed] Non-surgical pocket therapy: mechanical, pharmacotherapeutics, and dental occlusion. Journal of the American Dental Association (1939). 1998;129 Suppl:34S-9S.	Excluded by abstract
31	Chung CH, Vanarsdall RL, Cavalcanti EA, Baldinger JS, Lai CH. Comparison of microbial composition in the subgingival plaque of adult crowded versus non-crowded dental regions. The International journal of adult orthodontics and orthognathic surgery. 2000;15(4):321-30. Epub 2001/04/20.	Excluded by abstract
32	Costa MR, da Silva VC, Miqui MN, Colombo AP, Cirelli JA. Effects of ultrasonic, electric, and manual toothbrushes on subgingival plaque composition in orthodontically banded molars.Am J Orthod Dentofac Orthop 2010;137(2):229-35. Epub 2010/02/16.	Excluded by abstract
33	Davis SM, Plonka AB, Fulks BA, Taylor KL, Bashutski J. Consequences of orthodontic treatment on periodontal health: Clinical and microbial effects. Seminars in Orthodontics. 2014;20(3):139-49.	Excluded by abstract
34	Finkbeiner RL, Nelson LS, Killebrew J. Accidental orthodontic elastic band-induced periodontitis: orthodontic and laser treatment. Journal of the American Dental Association (1939). 1997;128(11):1565-9. Epub 1997/11/22.	Excluded by abstract
35	Frank CA, Long M. Periodontal concerns associated with the orthodontic treatment of impacted teeth.Am J Orthod Dentofac Orthop 2002;121(6):639-49. Epub 2002/06/25.	Excluded by abstract
36	Kurtulus Waschulewski I, Gokbuget AY, Christiansen NM, Ziegler M, Schuster V, Wahl G, et al. Immunohistochemical analysis of the gingiva with periodontitis of type I plasminogen deficiency compared to gingiva with gingivitis and periodontitis and healthy gingiva. Arch Oral Biol. 2016;72:75-86.	Excluded by abstract
37	Lewis EA, Ogle RE, Sorensen SE, Zysik DA. Clinical and laboratory evaluation of visible light-cured denture base resins and their application to orthodontics.Am J Orthod Dentofac Orthop 1988;94(3):207-15. Epub 1988/09/01.	Excluded by abstract
38	Qin F, Ma XQ, Xu PC. [Clinical study of premolar extrusion with subgingival decayed defect by one-couple orthodontic NiTi auxiliary arch]. Shanghai Kou Qiang Yi Xue. 2017;26(1):115-7.	Excluded by abstract
Check of full texts against the eligibility criteria		
39	Belludi SA, Deshpande RN, Belludi A, Byakod G. Changes in oral microbial flora and gingiva associated with fixed orthodontic therapy. International Journal of Clinical Dentistry. 2011;4(2):147-59.	Excluded; missing full-text
40	Du J, Xie YY. [The changes of subgingival microbials during periodontal maintenance in patients with gingivitic and wearing fixed orthodontic appliances]. Zhonghua kou qiang yi xue za zhi = Zhonghua kouqiang yixue zazhi = Chinese journal of stomatology. 2004;39(4):324-6. Epub 2004/09/30.	Excluded; missing full-text
41	Flores de Jacoby L, Muller HP. [Composition of the subgingival oral flora in wearers of removable orthodontic appliances]. Deutsche zahnarztliche Zeitschrift. 1982;37(11):925-8. Epub 1982/11/01. Zusammensetzung der subgingivalen Mundflora bei Tragern abnehmbarer kieferorthopadischer Gerate.	Excluded; missing full-text
42	Giordano J, Syed S, Caffesse R. Subgingival Plaque Flora of Children Undergoing Orthodontic Treatment. Journal of Dental Research. 1987;66:313-.	Excluded; missing full-text
43	Gusberti F, Kipioti A, Lang NP. The influence of orthodontal bands on subgingival microflora - A clinical longitudinal study. Schweizerische Monatsschrift fur Zahnmedizin [Internet]. 1983; (3):[591 p.].	Excluded; missing full-text
44	Mashimo PA, Hausmann E, Staple PH. Subgingival Microflora in Orthodontic Patients. Journal of Dental Research. 1980;59:515-.	Excluded; missing full-text
45	Muller HP, Flores de Jacoby L. [Composition of the oral subgingival flora in wearers of fixed orthodontic appliances]. Deutsche zahnarztliche Zeitschrift. 1982;37(10):855-60. Epub 1982/10/01. Zusammensetzung der subgingivalen Mundflora bei Tragern festsitzender kieferorthopadischer Gerate.	Excluded; missing full-text
46	Paolantonio M, Fanali S, Nuzzoli A, Di Girolamo G. [The prognostic significance of the presence of Actinobacillus actinomycetemcomitans in the subgingival plaque of young orthodontic patients]. Minerva stomatologica. 1995;44(5):195-203. Epub 1995/05/01. Significato prognostico della presenza di Actinobacillus actinomycetemcomitans nella placca sottogengivale di giovani pazienti ortodontici.	Excluded; missing full-text
47	Piccolomini R, Di Bonaventura G, Catamo G, Picciani C, Paolantonio M. Frequency of detection of Actinobacillus actinomycetemcomitans in young patients during fixed orthodontic therapy. The new microbiologica. 1996;19(4):345-9.	Excluded; missing full-text
48	Purucker P, Semrau K, Miethke RR, Bernimoulin JP. [Effect of various orthodontic retention elements on the composition of subgingival microflora]. Deutsche zahnarztliche Zeitschrift. 1987;42(5):458-62. Epub 1987/05/01. Einfluss verschiedener kieferorthopadischer Retentionselemente auf die Zusammensetzung der subgingivalen Mikroflora.	Excluded; missing full-text
49	Strohmenger L, Soragna I, Onofri M, Cerati M, Carrassi A. [Composition of subgingival bacterial plaque in subjects undergoing orthodontic treatment]. Mondo ortodontico. 1986;11(2):35-41. Epub 1986/03/01. Composizione della placca batterica subgengivale nei soggetti sottoposti a terapia ortodontica.	Excluded; missing full-text
50	Zhao H, Xie Y, Meng H. [Effect of fixed appliance on periodontal status of patients with malocclusion]. Zhonghua kou qiang yi xue za zhi = Zhonghua kouqiang yixue zazhi = Chinese journal of stomatology. 2000;35(4):286-8. Epub 2002/01/10.	Excluded; missing full-text
51	Freitas AO, Marquezan M, Nojima Mda C, Alviano DS, Maia LC. The influence of orthodontic fixed appliances on the oral microbiota: a systematic review. Dental Press J Orthod. 2014 Mar-Apr;19(2):46-55.	Excluded; systematic review
52	Gkantidis N, Christou P, Topouzelis N. The orthodontic-periodontic interrelationship in integrated treatment challenges: a systematic review. J Oral Rehabil. 2010 May 1;37(5):377-90.	Excluded; systematic review
53	Guo R, Lin Y, Zheng Y, Li W. The microbial changes in subgingival plaques of orthodontic patients: A systematic review and meta-analysis of clinical trials. BMC Oral Health. 2017;17(1).	Excluded; systematic review
54	Li LW, Wong HM, Sun L, Wen YF, McGrath CP. Anthropometric measurements and periodontal diseases in children and adolescents: a systematic review and meta-analysis. Adv Nutr. 2015;6(6):828-41.	Excluded; review
55	Maia LP, Novaes Jr AB, Souza SLSd, Palioto DB, Taba Jr M, Grisi MFdM. Ortodontia e periodontia – parte I: alterações periodontais após a instalação de aparelho ortodôntico [Orthodontics and periodontics: periodontal changes after the installation of orthodontic appliances]. Periodontia.21(3):40-5.	Excluded; review
56	Osmolska-Bogucka AE, Zadurska M. Microbial changes in subgingival plaque during orthodontic treatment – Literature review. Dental and Medical Problems. 2016;53(1):118-24.	Excluded; review
57	Jewtuchowicz VM, Brusca MI, Mujica MT, Gliosca LA, Finquelievich JL, Lovannitti CA, et al. Subgingival distribution of yeast and their antifungal susceptibility in immunocompetent subjects with and without dental	Excluded; no orthodontic treatment

	devices. Acta odontologica latinoamericana : AOL. 2007;20(1):17-22. Epub 2007/12/01.	
58	Gong Y, Lu J, Ding X. Clinical, microbiologic, and immunologic factors of orthodontic treatment-induced gingival enlargement.Am J Orthod Dentofac Orthop 2011;140(1):58-64.	Excluded; assessed changes by perio-tx
59	Bergamo AZN, Nelson P, Andrucioli MCD, do Nascimento C, Pedrazzi V, Matsumoto MAN. Microbial complexes levels in conventional and self-ligating brackets. Clinical Oral Investigations. 2017;21(4):1037-46.	Excluded; no subgingival microbiota measurement
60	Torlakovic L, Paster BJ, Ogaard B, Olsen I. Changes in the supragingival microbiota surrounding brackets of upper central incisors during orthodontic treatment. Acta Odontologica Scandinavica. 2013;71(6):1547-54.	Excluded; no subgingival microbiota measurement
61	Kim K, Jung WS, Cho S, Ahn SJ. Changes in salivary periodontal pathogens after orthodontic treatment: An in vivo prospective study. Angle Orthod. 2015 Nov 25. [Epub ahead of print]	Excluded; no subgingival microbiota measurement
62	Levrini L, Abbate GM, Migliori F, Orrù G, Sauro S, Caprioglio A. Assessment of the periodontal health status in patients undergoing orthodontic treatment with fixed or removable appliances. A microbiological and preliminary clinical study. Cumhuriyet Dental Journal. 2013;16(4):296-307.	Excluded; no subgingival microbiota found for analysis
63	Abbate GM, Caria MP, Montanari P, Mannu C, Orrù G, Caprioglio A, et al. Periodontal health in teenagers treated with removable aligners and fixed orthodontic appliances. Journal of Orofacial Orthopedics. 2015;76(3):240-50.	Excluded; no subgingival microbiota found for analysis
64	Demling A, Demling C, Schwestka-Polly R, Stiesch M, Heuer W. Short-Term Influence of Lingual Orthodontic Therapy on Microbial Parameters and Periodontal Status A Preliminary Study. Angle Orthodontist. 2010;80(3):480-4.	Excluded; samples taken from both treated (lower arch) and untreated sites (upper arch)
65	Di Murro C, Paolantonio M, Petti S, Tomassini E, Festa F, Grippaudo C, et al. [The clinical and microbiological evaluation of the efficacy of oral irrigation on the periodontal tissues of patients wearing fixed orthodontic appliances]. Minerva stomatologica. 1992;41(11):499-506. Epub 1992/11/01. Valutazione clinica e microbiologica sull'efficacia dell'irrigazione orale in tessuti parodontali di pazienti portatori di apparecchiature ortodontiche fisse.	Excluded; partial appliances
66	Diamanti-Kipioti A, Gusberti FA, Lang NP. Clinical and microbiological effects of fixed orthodontic appliances. Journal of clinical periodontology. 1987;14(6):326-33. Epub 1987/07/01.	Excluded; partial appliances
67	Perinetti G, Paolantonio M, Serra E, D'Archivio D, D'Ercole S, Festa F, et al. Longitudinal monitoring of subgingival colonization by Actinobacillus actinomycetemcomitans, and crevicular alkaline phosphatase and aspartate aminotransferase activities around orthodontically treated teeth. Journal of clinical periodontology. 2004;31(1):60-7.	Excluded; partial appliances
68	Demling A, Heuer W, Elter C, Heidenblut T, Bach FW, Schwestka-Polly R, et al. Analysis of supra- and subgingival long-term biofilm formation on orthodontic bands. European journal of orthodontics. 2009;31(2):202-6. Epub 2009/03/24.	Excluded; comparison of subgingival to supragingival plaque
69	Kim K, Heimisdottir K, Gebauer U, Persson GR. Clinical and microbiological findings at sites treated with orthodontic fixed appliances in adolescents.Am J Orthod Dentofac Orthop 2010;137(2):223-8. Epub 2010/02/16.	Excluded; different sites within patient compared
70	Paolantonio M, Pedrazzoli V, di Murro C, di Placido G, Picciani C, Catamo G, et al. Clinical significance of Actinobacillus actinomycetemcomitans in young individuals during orthodontic treatment. A 3-year longitudinal study. Journal of clinical periodontology. 1997;24(9 Pt 1):610-7.	Excluded; only comparisons among within-treatment timepoints
71	Lee SM, Yoo SY, Kim HS, Kim KW, Yoon YJ, Lim SH, et al. Prevalence of putative periodontopathogens in subgingival dental plaques from gingivitis lesions in Korean orthodontic patients. Journal of microbiology (Seoul, Korea). 2005;43(3):260-5.	Excluded; cross-sectional assessment
72	Liu P, Liu Y, Wang J, Guo Y, Zhang Y, Xiao S. Detection of fusobacterium nucleatum and fadA adhesin gene in patients with orthodontic gingivitis and non-orthodontic periodontal inflammation. PloS one. 2014;9(1):e85280.	Excluded; cross-sectional assessment
73	Paolantonio M, di Girolamo G, Pedrazzoli V, di Murro C, Picciani C, Catamo G, et al. Occurrence of Actinobacillus actinomycetemcomitans in patients wearing orthodontic appliances. A cross-sectional study. Journal of clinical periodontology. 1996;23(2):112-8.	Excluded; cross-sectional assessment
74	Pejda S, Varga ML, Milosevic SA, Mestrovic S, Slaj M, Repic D, et al. Clinical and microbiological parameters in patients with self-ligating and conventional brackets during early phase of orthodontic treatment. The Angle orthodontist. 2013;83(1):133-9.	Excluded; cross-sectional assessment
75	Rego RO, Oliveira CA, dos Santos-Pinto A, Jordan SF, Zambon JJ, Cirelli JA, et al. Clinical and microbiological studies of children and adolescents receiving orthodontic treatment. American journal of dentistry. 2010;23(6):317-23.	Excluded; cross-sectional assessment
76	Sargolzaie N, Amel-Jamedar S, Mokhtari MR, Arab HR, Piroozi S. Evaluation of Subgingival Dental Plaque Microbiota Changes In Fixed Orthodontic Patients with Syber Green Real Time PCR. Journal of Dental Materials and Techniques 2014;3(3):123-127.	Excluded; cross-sectional assessment
77	Vizitiu TC, Giuca MC, Ionescu E. Influence of orthodontic treatment on oral streptococci. Roumanian archives of microbiology and immunology. 2011;70(3):105-8.	Excluded; cross-sectional assessment
78	Bamani MSA, Shafshak S, MS. Microbiological and Gingival Tissue Changes associated with the Active Orthodontic Therapy. Journal of Applied Sciences Research 2013;9(9):5489-5496.	Excluded; no quantitative data
79	Folco AA, Benítez Rogé SC, Iglesias M, Calabrese D, Pelizardi C, Rosa de Nastri AC, et al. Gingival response in orthodontic patients: comparative study between self-ligating and conventional brackets. Acta odontologica latinoamericana : AOL.27(3):120-4.	Excluded; no quantitative data
80	Ireland AJ, Soro V, Sprague SV, Harradine NWT, Day C, Al-Anezi S, et al. The effects of different orthodontic appliances upon microbial communities. Orthodontics & craniofacial research. 2014;17(2):115-23.	Excluded; no quantitative data
81	Karkhanechi M, Chow D, Sipkin J, Sherman D, Boylan RJ, Norman RG, Craig RG, Cisneros GJ. Periodontal status of adult patients treated with fixed buccal appliances and removable aligners over one year of active orthodontic therapy. Angle Orthod. 2013 Jan;83(1):146-51.	Excluded; no quantitative data
82	Levrini L, Mangano A, Montanari P, Margherini S, Caprioglio A, Abbate GM. Periodontal health status in patients treated with the Invisalign(®) system and fixed orthodontic appliances: A 3 months clinical and microbiological evaluation. Eur J Dent. 2015 Jul-Sep;9(3):404-10.	Excluded; no quantitative data
83	Sinclair PM, Berry CW, Bennett CL, Israelson H. Changes in gingiva and gingival flora with bonding and banding. Angle Orthod. 1987 Oct;57(4):271-8.	Excluded; no quantitative data
84	Speer C, Pelz K, Hopfenmuller W, Holtgrave EA. Investigations on the influencing of the subgingival microflora in chronic periodontitis. A study in adult patients during fixed appliance therapy. J Orofac Orthop 2004;65(1):34-47.	Excluded; no quantitative data
	Included	

85	Amasyali M, Enhos S, Uysal T, Saygun I, Kilic A, Bedir O. Effect of a self-etching adhesive containing an antibacterial monomer on clinical periodontal parameters and subgingival microbiologic composition in orthodontic patients.Am J Orthod Dentofac Orthop 2011;140(4):e147-53.	Included
86	Choi DS, Cha BK, Jost-Brinkmann PG, Lee SY, Chang BS, Jang I, et al. Microbiologic changes in subgingival plaque after removal of fixed orthodontic appliances. The Angle orthodontist. 2009;79(6):1149-55.	Included
87	Demling A, Demling C, Schwestka-Polly R, Stiesch M, Heuer W. Influence of lingual orthodontic therapy on microbial parameters and periodontal status in adults. European journal of orthodontics. 2009;31(6):638-42.	Included
88	Ghijselings E, Coucke W, Verdonck A, Teughels W, Quirynen M, Pauwels M, et al. Long-term changes in microbiology and clinical periodontal variables after completion of fixed orthodontic appliances. Orthodontics & craniofacial research. 2014;17(1):49-59.	Included
89	Guo L, Feng Y, Guo HG, Liu BW, Zhang Y. Consequences of orthodontic treatment in malocclusion patients: Clinical and microbial effects in adults and children. BMC Oral Health. 2016;16(1).	Included
90	Hassan KS, Alagl AS, Ali I. Periodontal status following self-ligature versus archwire ligation techniques in orthodontically treated patients—Clinical, microbiological and biochemical evaluation. Orthod Waves 2010;69:164-170.	Included
91	Kim SH, Choi DS, Jang I, Cha BK, Jost-Brinkmann PG, Song JS. Microbiologic changes in subgingival plaque before and during the early period of orthodontic treatment. The Angle orthodontist. 2012;82(2):254-60.	Included
92	Leung NM, Chen R, Rudney JD. Oral bacteria in plaque and invading buccal cells of young orthodontic patients.Am J Orthod Dentofac Orthop 2006;130(6):698 e11-8.	Included
93	Liu H, Sun J, Dong Y, Lu H, Zhou H, Hansen BF, et al. Periodontal health and relative quantity of subgingival Porphyromonas gingivalis during orthodontic treatment. The Angle orthodontist. 2011;81(4):609-15.	Included
94	Lo BA, Di Marco R, Milazzo I, Nicolosi D, Cali G, Rossetti B, et al. Microbiological and clinical periodontal effects of fixed orthodontic appliances in pediatric patients. The new microbiologica. 2008;31(2):299-302.	Included
95	Lu H, Zhou HM, Song XB, Sun JL, Liu HY. [Quantitative study of porphyromonas gingivalis in subgingival plaques of orthodontic adults]. Hua xi kou qiang yi xue za zhi = Huaxi kouqiang yixue zazhi = West China journal of stomatology. 2010;28(2):166-9.	Included
96	Martha K, Lorinczi L, Bica C, Gyergyay R, Petcu B, Lazar L. Assessment of Periodontopathogens in Subgingival Biofilm of Banded and Bonded Molars in Early Phase of Fixed Orthodontic Treatment. Acta Microbiol Immunol Hung. 2016;63(1):103-13.	Included
97	Menezes Cardoso R. Evaluation of status and gingival subgingival microbial of periodontally healthy patients undergoing treatment orthodontic. Dissertation, Universidade Federal de Pernambuco, 2011.	Included
98	Montaldo C, Erriu M, Giovanna Pili FM, Peluffo C, Nucaro A, Orrù G, et al. Microbial changes in subgingival plaque and polymicrobial intracellular flora in buccal cells after fixed orthodontic appliance therapy: A preliminary study. International Journal of Dentistry. 2013;2013.	Included
99	Naranjo AA, Trivino ML, Jaramillo A, Betancourth M, Botero JE. Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement.Am J Orthod Dentofac Orthop 2006;130(3):275 e17-22.	Included
100	Paolantonio M, Festa F, di Placido G, D'Attilio M, Catamo G, Piccolomini R. Site-specific subgingival colonization by Actinobacillus actinomycetemcomitans in orthodontic patients.Am J Orthod Dentofac Orthop 1999;115(4):423-8.	Included
101	Petti S, Barbato E, Simonetti D'Arca A. Effect of orthodontic therapy with fixed and removable appliances on oral microbiota: a six-month longitudinal study. The new microbiologica. 1997;20(1):55-62. Epub 1997/01/01.	Included
102	Ristic M, Vlahovic Svabic M, Sasic M, Zelic O. Clinical and microbiological effects of fixed orthodontic appliances on periodontal tissues in adolescents. Orthodontics & craniofacial research. 2007;10(4):187-95.	Included
103	Ristic M, Vlahovic Svabic M, Sasic M, Zelic O. Effects of fixed orthodontic appliances on subgingival microflora. International journal of dental hygiene. 2008;6(2):129-36.	Included
104	Sallum EJ, Nouer DF, Klein MI, Goncalves RB, Machion L, Sallum AW, et al. Clinical and microbiologic changes after removal of orthodontic appliances. American Journal of Orthodontics and Dentofacial Orthopedics. 2004;126(3):363-6.	Included
105	Sandic MZ, Popovic B, Carkic J, Nikolic N, Glisic B. Changes in subgingival microflora after placement and removal of fixed orthodontic appliances. Srpski arhiv za celokupno lekarstvo. 2014;142(5-6):301-5.	Included
106	Shi J, Liu Y, Hou J, Yan Z, Peng H, Chang X. [Comparison of periodontal indices and Porphyromonas gingivalis between conventional and self-ligating brackets]. Hua Xi Kou Qiang Yi Xue Za Zhi. 2013 Jun;31(3):228-31.	Included
107	Thornberg MJ, Riolo CS, Bayirli B, Riolo ML, Van Tubergen EA, Kulbersh R. Periodontal pathogen levels in adolescents before, during, and after fixed orthodontic appliance therapy. Am J Orthod Dentofacial Orthop. 2009 Jan;135(1):95-8.	Included
108	van Gastel J, Quirynen M, Teughels W, Coucke W, Carels C. Longitudinal changes in microbiology and clinical periodontal parameters after removal of fixed orthodontic appliances. European journal of orthodontics. 2011;33(1):15-21.	Included
109	van Gastel J, Quirynen M, Teughels W, Coucke W, Carels C. Longitudinal Changes in Microbiology and Clinical Periodontal Variables After Placement of Fixed Orthodontic Appliances. Journal of periodontology. 2008;79(11):2078-86.	Included
110	van Gastel J, Teughels W, Quirynen M, Struyf S, Van Damme J, Coucke W, et al. Longitudinal changes in gingival crevicular fluid after placement of fixed orthodontic appliances.Am J Orthod Dentofac Orthop 2011;139(6):735-44.	Included
111	van Gastel J. Periodontal and microbial implications of orthodontic treatment with fixed appliances. Dissertation: Catholic University of Leuve, 2009.	Included
112	Yáñez-Vico RM, Iglesias-Linares A, Ballesta-Mudarra S, Ortiz-Ariza E, Solano-Reina E, Perea EJ. Short-term effect of removal of fixed orthodontic appliances on gingival health and subgingival microbiota: A prospective cohort study. Acta Odontologica Scandinavica. 2015;73(7):496-502.	Included
113	Yang YM, Kim SS, Jun ES, Park SB. Changes of periodontopathogens and clinical parameters of periodontal tissue after debanding. Korean J Orthod 2006 Aug 36(4):263-274.	Included

Tx, treatment.

Appendix 6a. Characteristics of the included trials (design, patient, and treatment characteristics).

No	Study ID	Design; setting; country[£]	Patient (M/F)	Age*	Intervention	Br/Bd	Ligation	Tx Jaw
1	Amasyali 2011	smRCT; Uni; TR	Exp1/Exp2: 15 (7/8)	Exp1/Exp2: 14.4	Exp1: FA Exp2: FA antibacterial	Br	NR	Both
2	Choi 2009	uCCT; Uni; KR	Exp: 30 (11/19) Ctr: 30 (13/17)	Exp: 20.0 Ctr: 16.7	FA	Br/Bd	NR	Both
3	Demling 2009	uCCT; Uni; DE	Exp: 10 (2/8)	Exp: 29.0	FA (lingual)	Br	Elastic/SS	Both
4	Guo 2016	uCCT; Uni; CN	Exp1: 62 (NR) Exp2: 46 (NR)	Exp1: (8.0-15.0) Exp2: (18.0-32.0)	FA	Br/Bd	NR	Both
5	Hassan 2010	smRCT; Uni; SA	Exp1/Exp2: 22 (10/12)	Exp1/Exp2: 17.1	Exp1: FA Exp2: FA (SL)	Br	Exp1: NR Exp2: None	Both
6	Kim 2012	uCCT; Uni; KR	Exp: 30 (13/17)	Exp: 16.7	FA	Br/Bd	SS	Both
7	Leung 2006	uCCT; Uni; US	Exp: 27 (14/13)	Exp: 14.9	FA	NR	NR	Both
8	Liu 2011; pt 1	uCCT; Uni; CN	Exp: 28 (6/22)	Exp: 17.6	FA	Br/Bd	NR	Both
8	Liu 2011; pt 2	uCCT; Uni; CN	Exp: 20 (7/13)	Exp: 17.8	FA	Br/Bd	NR	Both
9	Lo 2008	pCCT; Uni; IT	Exp: 10 (4/6)	Exp: 13.1	FA	NR	NR	NR
10	Lu 2010	uCCT; Uni; CN	Exp: 11 (2/9)	Exp: 22.5	FA	Br/Bd	NR	Both
11	Martha 2016	uCCT; Uni; RO	Exp1: 15 (NR) Exp2: 10 (NR)	Exp1: 14.4 Exp2: 15.7	Exp1/2: FA	Exp1: Br/Bd Exp2: Br	Exp1/2: NR	Exp1/2: Both
12	Menezes Cardoso 2011	uCCT; Uni; BR	Exp: 10 (2/8) Ctr: 10 (6/4)	Exp/Ctr: up to 18.0	FA	Br/Bd	NR	Both
13	Montaldo 2013	uCCT; Uni; IT	Exp: 19 (7/12)	Exp: 13.3	FA	NR	NR	Both
14	Naranjo 2006	uCCT; Uni; CO	Exp: 30 (12/18)	Exp: 18.7	FA	NR	NR	Both
15	Paolantonio 1999	smRCT; Uni; IT	Exp/Ctr: 24 (11/13)	Exp/Ctr: 18.0-22.0	FA	Br/Bd	NR	Max or Mand
16	Petti 1997	uCCT; Uni; IT	Exp1: 15 (NR) Exp2: 15 (NR)	Exp1/2: (7.0-15.0)	Exp1: FA Exp2: RA	NR	Exp1/2: NR	Exp1/2: NR
17	Ristic 2007;2008	pCCT; Uni; RS	Exp: 32 (13/19)	Exp: 12.0-18.0	FA	Br/Bd	NR	Both
18	Sallum 2004	pCCT; Uni; BR	Exp: 10 (4/6)	Exp: 16.0	FA	NR	NR	NR
19	Sandic 2014 pt1	uCCT; Uni; RS	Exp: 33 (14/19)	Exp: 19.7	FA	NR	NR	Max
19	Sandic 2014 pt2	uCCT; Uni; RS	Exp: 33 (14/19)	Exp: 19.7	FA	NR	NR	Max
20	Shi 2013	uCCT; Uni; CN	Exp1: 15 (6/9) Exp2: 15 (8/7)	Exp1: 15.8 Exp2: 14.5	Exp1: FA Exp2: FA (SL)	NR	Exp1: NR Exp2: None	Both
21	Thornberg 2009	uCCT; Uni; US	Exp: 190 (89/101)	Exp: 13.5	FA	NR	NR	Both
22	van Gastel 2008†	pCCT; Uni; BE	Exp: 24 (10/14)	Exp: 14.6	Exp1: FA Exp2: Bds+HG and FA	Br/Bd	Exp1/Exp2: Elastics	Exp1/Exp2: Both
23	Yanez-Vico 2015	pCCT; Uni; ES	Exp: 61 (NR) Ctr: 61 (NR)	Exp/Ctr: 21.3	Exp: FA	Br/Bd	NR	Both
24	Yang 2006	uCCT; Uni; KR	Exp: 17 (6/11)	Exp: 22.0	FA	Br/Bd	NR	Both

[£] countries are given with their ISO alpha-2 codes

* patient ages are reported as means (one value) or if no mean is available as range (two values in parenthesis)
† collated papers include van Gastel 2008, van Gastel 2009, van Gastel 2011a, van Gastel 2011b, and Ghijselings 2014
Bd, band; Br, bracket (or bonded attachments for molars); Ctr, control group; Exp, experimental group; FA, fixed appliance (labial and conventionally ligated, unless otherwise noted); HG, headgear; M/F, male/female; NR, not reported; pCCT, prospective non-randomized controlled clinical trial; RA, removable appliance; SL, self-ligated; smRCT, split-mouth randomized clinical trial; SS, stainless steel; uCCT, non-randomized controlled clinical trial with unclear design; Uni; university.

Appendix S6b. Characteristics of the included trials (outcome details)/

No	Study ID	Sampled teeth (FDI)	Timing	Outcome
1	Amasyali 2011	15,12,22,25,35, 32,42,45	T0: before AppIns T1: 6 mos after T0	RT-PCR (qual/quant): Pg, Tf, Pi, Aa, Fn, Cr
2	Choi 2009	21,26,36,31	T0: 2 wks before AppRem T1: 3 mos after AppRem	PCR (qual): Aa, Tf, Cr, Ec, Pg, Pi, Pn, Td
3	Demling 2009	16,14,11,36,34, 31 (or 26,24,21, 41,44, 46)	T0: before AppIns T1: 3 mos after T0	PCR (qual): Aa, Pg
4	Guo 2016	35,34,31,41,44, 45	T0: before AppIns T1: 1 mo after T0 T2: 3 mos after T0	PCR (qual): Fn, Pg, Pi, Tf
5	Hassan 2010	13,23,33,43	T0: before AppIns T1: 1 wk after T0 T2: 1 mo after T0 T3: 3 mos after T0 T4: 6 mos after T0	Culture (quant): total bacteria, anaerobic lactobacilli, aerobic lactobacilli, Sm
6	Kim 2012	21,26,36,31	T0: before AppIns T1: 1 wk after T0 T2: 3 mos after T0 T3: 6 mos after T0	PCR (qual): Aa, Tf, Cr, Ec, Pg, Pi, Pn, Td
7	Leung 2006	15,25,35,45	T0: before AppIns T1: 7 wks after T0	DNA quantitation: bacterial DNA PCR (quant): total bacteria, streptococci, Aa, Tf
8	Liu 2011	42,41,31,32	T0: before AppIns T1: 1 mo after T0 T2: 3 mos after T0	RT-PCR (qual/quant): Pg; total bacteria
8	Liu 2011	42,41,31,32	T0: before AppRem T1: 1 mo after T0 T2: 3 mos after T0 T3: 6 mos after T0	RT-PCR (qual/quant): Pg; total bacteria
9	Lo 2008	16,26,36,46	T0: before AppIns T1: 2 wks after T0 T2: 4 wks after T0 T3: 12 wks after T0	Microbiology NR: frequency of multiple bacteria; % aerobic/anaerobic bacteria
10	Lu 2010	31,41	T0: before AppIns T1: 1 mo after T0 T2: 3 mos after T0	RT-PCR (qual/quant): total bacteria, Pg
11	Martha 2016	16,26,36,46	T0: before AppIns T1: 1-1.6 mo after T0	PCR (qual): Aa, Cr, Csp, En, Ec, Fn, Pg, Pi, Pm, Td, Tf; total positive sites
12	Menezes Cardoso 2011	16,11,36,31	T0: before AppIns T1: 3 mos after T0 T2: 6 mos after T0	PCR (qual): Aa, Tf
13	Montaldo 2013	15,25	T0: before AppIns T1: 1 mo after T0 T2: 2 mos after T0 T3: 3 mos after T0	PCR (qual): Aa, Tf, Pg, Pi, Td
14	Naranjo 2006	15,12,22,25,35, 45	T0: before AppIns T1: 3 mos after T0	Culture (qual): Pg, Tf, Pi, Pn, Fs, Pm, Campylobacters, Eubacteriums, Aa, Ec, G- rods and their virulence factors, Capnocytophagae, Dp, β-hemolytic streptococci, Staphylococci, yeasts
15	Paolantonio 1999	16,12,22,26,36, 32,42,46	T0: before AppIns T1: 4 wks after T0 T2: 8wks after T0 T3: 12 wks after T0; AppRem T4: 4 wks after T3	Culture (qual/quant): Aa, total anaerobic
16	Petti 1997	16,26,36,46	T0: before AppIns T1: 1.5-2.0 mos after T0 T2: 6.0-7.0 mos after T0	PCR (qual): Aa, Pg, G+ cocci, G- rods, motile rods, spirochetes PCR (quant): total bacterial count
17	Ristic 2007;2008	16,21,24	T0: before AppIns T1: 1 mo after T0 T2: 3 mos after T0 T3: 6 mos after T0	Culture: bacterial composition; frequency of Pi, Aa, pigmented anaerobes
18	Sallum 2004	16, 11, 26	T0: before AppRem T1: 1 mo after T0	PCR (qual): Pg, Bf, Aa, Pi, Pn
19	Sandic 2014 pt1	16,11	T0: before AppIns T1: 1 mo after T0 T2: 3 mos after T0	PCR (qual): Aa, Pg, Tf, Pi
19	Sandic 2014 pt2	16,11	T0: before AppRem T1: 1 mo after T0	PCR (qual): Aa, Pg, Tf, Pi

			T2: 3 mos after T0	
20	Shi 2013	31,41	T0: before AppIns T1: 1 mo after T0 T2: 3 mos after T0	PCR (quant): total bacteria, Pg
21	Thornberg 2009	16,11,24,36,31, 44	T0: before AppIns T1: 6 mos after T0 T2: 12 mos after T0 T3: more than 12 mos after T0 T4: 3 mos after AppRem	DNA probe technique (qual): Aa, Pg, Pi, Tf, Ec, Fn, Td, Cr
22	van Gastel 2008	16,14	T0: band placement (Exp2) T1: FA placement (Exp1 anc Exp2) T2: 1 yr after T1 T3: AppRem (21.0 mos after T1) T4: 3 mos after T1 T5: 2 yrs after T1	Culture: CFU aerobe/anaerobe; GCF flow; prevalence of Pg, Pi, and other
23	Yanez-Vico 2015	15, 14, 11, 34, 45	T0: before AppRem (31.6 mos after insertion) T1: 10 d after T0	PCR (qual): Aa, Pg, Pi, Tf, Td
24	Yang 2006	16,26,36,46 (or 17,27,37,47)	T0: before AppIns T1: 4 wks after T0	PCR (qual): Pg, Tf, Td

*patient ages are reported as means (1 value) or if no mean is available as range (2 values in parenthesis)
† collated papers include van Gastel 2008, van Gastel 2009, van Gastel 2011a, van Gastel 2011b, and Ghijselings 2014
Aa, Aggregatibacter actinomycetemcomitans; AppIns, appliance insertion; AppRem, appliance removal; Bf, Bacteroides forsythus; CFU, Colony forming units; Cr, Campylobacter rectus; Csp, Capnocytophaga spp; d, day; Dp, Dialister pneumosintes; Ec, Eikenella corrodens; En, Eubacterium nodatum; FDI, federation dentaire international; Fn, Fusobacterium nucleatum; Fs, Fusobacterium species; G-, Gram-negative; G+, Gram-positive; GCF, gingival crevicular fluid; mo, month; PCR, polymerase chain reaction; Pg, Porphyromonas gingivalis; Pi, Prevotella intermedia; Pm, Parvimonas micra; Pn, Prevotella nigrescens; qual, qualitative; quant, quantitative; RT, real time; Sm, Streptococcus mutans; Td, Treponema denticola; Tf, Tannerella forsythia; Uni; university; wk, weeks; yr, year.

Appendix S7a. Details of the risk of bias assessment for included randomized controlled trials.

Trial	Sequence generation	Allocation concealment	Blinding of participants/ personnel	Blinding of outcome assessors	Incomplete outcome data	Selective outcome reporting	Other sources of bias	Overall
Amasyali 2011	High risk - quasi-randomization employed in a split-mouth non-randomized manner: "This study was organized with a split-mouth design with contralateral antagonistic quadrants receiving the experimental material and the control."	Unclear - no information provided.	Low risk - Blinding is impractical for both patients and clinician; outcome is objective and was assessed blindly.	Low risk - "This clinician was blinded to the group allocations".	Low risk - No drop-outs or patient losses are reported.	Unclear - It is difficult to judge whether selective reporting is a problem, as no protocol exists.	Unclear - residual bias cannot be excluded; systemic health and smoking status not reported; oral hygiene was taught.	High risk
Hassan 2010	Low risk -"Randomly selected, by the flip of a coin, the right side was ligated with self-ligature technique (0.022 in. Damon3TM,Ormco, Orange, CA, USA) while those on the left side with conventional stainless steel ligature wires, using brackets with hook".	Unclear - no information provided.	Unclear - Blinding is impractical for both patients and clinician; outcome is objective, but was not assessed blindly.	High risk - no mention of blinding throughout the paper; blinding could have been implemented.	Low risk - No drop-outs or patient losses are reported.	Unclear - It is difficult to judge whether selective reporting is a problem, as no protocol exists.	Unclear - residual bias cannot be excluded;smoking status not reported; oral hygiene was taught.	High risk
Paolantonio 1999	Unclear - unclear randomization: "After clinical and microbiologic examinations, the patients received fixed orthodontic appliances in only 1 randomly chosen dental arch (test sites)—the other was left free from appliances (control sites)".	Unclear - no information provided.	Unclear - Blinding is impractical for both patients and clinician; outcome is objective, but was not assessed blindly.	High risk - no mention of blinding throughout the paper; blinding could have been implemented.	Low risk - No drop-outs or patient losses are reported.	Unclear - It is difficult to judge whether selective reporting is a problem, as no protocol exists.	Unclear - residual bias cannot be excluded;smoking status not reported; oral hygiene was taught.	High risk

Appendix S7b. Details of the risk of bias assessment for included non-randomized controlled trials.

C	Category	Item	Choi 2009	Demling 2009	Guo 2016	Kim 2012	Leung 2006	Liu 2011	Lo 2008	Lu 2010	Martha 2016	Menezes Cardoso 2011	Montaldo 2013	Naranjo 2006	Petti 1997	Ristic 2007;2008	Sallum 2004	Sandic 2014 pt1	Shi 2013	Thornberg 2009	van Gastel 2009	Yanez-Vico 2015	Yang 2006
1	External validity	Was selection of Tx and Ctr patients drawn from the same population and over the same period?	Probably yes	Not applicable	Not applicable	Not applicable	Not applicable	Definitely yes	Not applicable	Not applicable	Not applicable	Definitely yes	Not applicable	Probably yes	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Definitely yes	Not applicable
2		Can we be confident that Tx patients were treated?	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes
3		Can we be confident that Ctr patients were not treated?	Definitely yes	Not applicable	Not applicable	Not applicable	Not applicable	Definitely yes	Not applicable	Not applicable	Not applicable	Definitely yes	Not applicable	Definitely yes	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Definitely yes	Not applicable
4		Can we be confident that periodontal health was not used as a patient selection criterion?	Probably yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Unclear	Probably yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely no	Unclear	Definitely yes	Unclear	Definitely yes	Definitely yes	Definitely yes
5	Internal validity	Are the characteristics of the patients included in the study clearly described ?	Definitely yes	Definitely yes	Probably yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Unclear	Definitely yes	Definitely yes	Unclear	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Unclear	Definitely yes
6		Did the study match Tx and Ctr patients for all variables that are associated with periodontal health (age, gender, smoking, diabetes) or did the statistical analysis adjust for these prognostic variables?	Probably yes	Not applicable	Not applicable	Not applicable	Not applicable	Unclear	Not applicable	Not applicable	Not applicable	Unclear	Not applicable	Probably yes	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Unclear	Not applicable
7		Can we be confident in the assessment of periodontal disease (outcome measures valid and reliable)?	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely yes
8		Was outcome measurement performed blinded?	Definitely no	Definitely no	Definitely yes	Definitely no	Definitely no	Definitely no	Definitely no	Definitely no	Definitely no	Definitely no	Definitely no	Definitely no	Definitely no	Definitely no	Definitely no	Definitely no	Definitely no	Definitely no	Definitely yes	Definitely no	Definitely no
9	Reporting	Is reporting of results complete and transparent (for continuous outcomes: n, mean, SD; for binary outcomes: sample and events; for calculated effect sizes: point estimate and SE or 95% CI)	Definitely yes	Definitely yes	Definitely yes	Definitely yes	Definitely no	Definitely no	Definitely no	Definitely yes	Definitely yes	Definitely yes	Definitely no	Probably no	Definitely yes	Definitely no	Definitely no	Definitely yes	Definitely no	Probably yes	Definitely yes	Definitely yes	Definitely yes
10		Were the statistical tests used to assess the main outcomes appropriate (normality/pairness)?	Probably no	Definitely no	Definitely no	Definitely yes	Definitely yes	Probably yes	Probably yes	Definitely yes	Probably no	Definitely yes	Probably no	Probably no	Probably no	Probably no	Unclear	Definitely yes	Probably yes	Probably yes	Definitely yes	Definitely yes	Definitely yes
11		Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	Definitely yes	Definitely yes	Definitely no	Definitely no	Definitely yes	Definitely no	Definitely no	Definitely no	Definitely yes	Definitely yes	Definitely no	Definitely no	Definitely yes	Definitely no	Definitely no	Definitely yes	Definitely no	Definitely no	Definitely no	Definitely yes	Definitely no

Legend

- Definitely yes
- Probably yes
- Unclear
- Probably no
- Definitely no
- Not applicable

C, Criterion; CI, confidence interval; Ctr, control; SD, standard deviation; SE, standard error; Tx, treatment.

*Appendix S8a***.** List of all extracted outcomes from included studies: detection of single periodontopathogens in the subgingival sulcus.

Study	**INS**	**REM**	**CTR**	**INT**	**outc**	**Aa**	**Ai**	**Am**	**Ao**	**Av**	**Bf**	**Cg**	**Cr**	**Ec**	**En**	**Fn**	**Mm**	**Pa**	**Pb**	**Pd**	**Pg**	**Pi**	**PRme**	**PAmi**	**Pn**	**Tf**	**Td**
Demling 2009	x			fix	0/1	bi															bi						
Kim 2012	x			fix	0/1	bi							bi	bi							bi	bi			bi	bi	bi
Leung 2006	x			fix	0/1	bi																				bi	
Liu 2011	x			fix	0/1																bi						
Lo 2008	x			fix	0/1	bi	bi	bi	bi	bi		bi		bi		bi	bi	bi	bi	bi	bi	bi	bi				
Lu 2010	x			fix	0/1																bi						
Martha 2016	x			fix	0/1	bi							bi	bi	bi	bi					bi	bi		bi		bi	bi
Menezes 2011	x		x	fix	0/1	bi																				bi	
Montaldo 2013	x			fix	0/1																					bi	
Paolantonio 1999	x		x	fix	0/1	bi																					
Petti 1997	x			fix	0/1	bi															bi						
Ristic 2007	x			fix	0/1	bi																					
Sandic 2014	x			fix	0/1	bi															bi	bi				bi	
Thornberg 2009	x			fix	0/1	bi							bi	bi		bi					bi	bi				bi	bi
Petti 1997	x			rem	bin	bi															1						
Amasyali 2011	x			fix	quant$_{median}$	co							con			con					con	con				con	
Guo 2016	x			fix	quant$_{mean}$											con					con	con				con	
Hassan 2010	x			fix	quant$_{CFU/ml}$																						
Lu 2010	x			fix	quant$_{\%proport.}$																con						
Naranjo 2006	x			fix	quant$_{\%proport.}$	con								con			con				con	con			con	con	
Petti 1997	x			fix	quant$_{\%proport.}$																						
Shi 2013	x			fix	quant$_{proport}$																con						
Petti 1997	x			rem	quant																						
Choi 2009		x	x	fix	0/1	bi							bi	bi							bi	bi			bi	bi	bi
Liu 2011		x		fix	0/1															bi	bi						
Paolantonio 1999		x	x	fix	0/1	bi																					
Sallum 2004		x		fix	0/1	bi					bi										bi	bi			bi		
Sandic 2014		x		fix	0/1	bi															bi	bi				bi	
Thornberg 2009		x		fix	0/1	bi							bi	bi		bi					bi	bi				bi	bi
Yang 2006		x		fix	0/1																bi					bi	bi
Yanez-Vico 2015		x	x	fix	0/1	bi															bi	bi				bi	bi
Thornberg 2009		x		fix	high	bi							bi	bi		bi					bi	bi				bi	bi

Ins, Insertion of appliances; REM, Removal of appliances; CTR, Control group; ANlac, anaerobic lactobacilli; AElac, aerobic lactobacilli; Sm, Streptococcus mutans; AST, Aspartate aminotransferase activity; bact, bacteria; camp, Campylobacter; capnof, Capnocytophaga spp.; cfurat, colony forming units ratio; eub, Eubacterium species; gmirod, Gram negative rods; gpococ, Gram positive cocci; strep, Streptococci; fusob, Fusobacterium species; piganabac, pigmented anaerobe bacteria; pseud, Pseudomonas species; rods, motile rods; spir, spirochetes; totpos, overall positive sites; INT, intervention type; outc, outcome; bi, binary outcome; con, continuous outcome; fix, fixed appliances; rem, removable appliances.

Appendix S8b. List of all extracted outcomes from included studies: cumulative measurements in the subgingival sulcus.

Study	Ins	REM	CTR	Total bacteria	ANlac	AElac	Sm	AST	bact	camp	capnof	cfurat	eub	gmirod	gpococ	strep	fusob	piganabac	pseud	rods	spir	totpos	INT	outc
Demling 2009	x		0																				fix	0/1
Kim 2012	x		0																				fix	0/1
Leung 2006	x		0						bi							bi							fix	0/1
Liu 2011	x		0																				fix	0/1
Lo 2008	x		0													bi			bi				fix	0/1
Lu 2010	x		0																				fix	0/1
Martha 2016	x		0								bi											bi	fix	0/1
Menezes 2011	x		1																				fix	0/1
Montaldo 2013	x		0																				fix	0/1
Paolantonio 1999	x		1																				fix	0/1
Petti 1997	x		0											bi	bi					bi	bi		fix	0/1
Ristic 2007	x		0															bi					fix	0/1
Sandic 2014	x		0																				fix	0/1
Thornberg 2009	x		0																				fix	0/1
Petti 1997	x		0											bi	bi					bi	bi		rem	bin
Amasyali 2011	x		0																				fix	quant
Guo 2016	x		0																				fix	quant
Hassan 2010	x		0	con	con	con	con	con															fix	quant
Lu 2010	x		0																				fix	quant
Naranjo 2006	x		0							con			con	con			con						fix	quant
Petti 1997	x		0						con														fix	quant
Shi 2013	x								con														fix	quant
van Gastel 2009	x											con											fix	quant
Petti 1997	x								con														rem	quant
Choi 2009		x	1																				fix	0/1
Liu 2011		x	0																				fix	0/1
Paolantonio 1999		x	1																				fix	0/1
Sallum 2004		x	0																				fix	0/1
Sandic 2014		x	0																				fix	0/1
Thornberg 2009		x	0																				fix	0/1
Yang 2006		x	0																				fix	0/1
Yanez-Vico 2015		x	1																				fix	0/1
Thornberg 2009		x	0																				fix	0/1

Ins, Insertion of appliances; REM, Removal of appliances; CTR, Control group; ANlac, anaerobic lactobacilli; AElac, aerobic lactobacilli; Sm, Streptococcus mutans; AST, Aspartate aminotransferase activity; bact, bacteria; camp, Campylobacter; capnof, Capnocytophaga spp.; cfurat, colony forming units ratio; eub, Eubacterium species; gmirod, Gram negative rods; gpococ, Gram positive cocci; strep, Streptococci; fusob, Fusobacterium species; piganabac, pigmented anaerobe bacteria; pseud, Pseudomonas species; rods, motile rods; spir, spirochetes; totpos, overall positive sites; INT, intervention type; outc, outcome; bi, binary outcome; con, continuous outcome; fix, fixed appliances; rem, removable appliances.

*Appendix S9a***.** Subgroup analyses and meta-regressions from uncontrolled studies of the effect of orthodontic appliance insertion on the subgingival microbiota of orthodontically treated patients.

	Prevalence of *A. a.*			Prevalence of *P. g.*			Prevalence of *P. i.*			Prevalence of *T. f.*		
Subgroup	**n**	**RR (95% CI)**	**P_{SG}**	**n**	**RR (95% CI)**	**P_{SG}**	**n**	**RR (95% CI)**	**P_{SG}**	**n**	**RR (95% CI)**	**P_{SG}**
Patient age	3	1.09 (0.14,8.32)	0.70	6	1.06 (0.88,1.28)	0.41	3	2.81 (0.02,471.43)	0.24	-	-	-
Male % of sample	4	1.05 (0.64,1.74)	0.69	5	1.01 (0.92,1.11)	0.78	3	2.11 (0.08,56.20)	0.21	-	-	-
FU < 3 mos	1	1.67 (0.46,6.07)	0.83	1	1.51 (0.27,8.29)	**0.09$**	1	1.00 (0.28,3.58)	0.72	2	1.49 (0.75,2.96)	0.17
3 mos ≤ FU < 6 mos	4	1.36 (0.19,9.98)		5	0.59 (0.36,0.97)		2	0.65 (0.15,2.78)		2	0.46 (0.03,7.52)	
FU ≥ 6 mos	3	2.04 (0.44,9.49)		2	1.80 (0.86,3.75)		2	2.03 (0.53,7.77)		2	**4.91 (2.56,9.40)***	
Lingual brackets	1	1.12 (0.40,3.15)	0.79	1	1.99 (0.21,18.62)	0.56	-	-	-	-	-	-
Conventional brackets	7	1.71 (0.50,5.81)		7	0.79 (0.46,1.35)		-	-	-	-	-	-
Patient level	6	2.39 (0.85,6.70)	0.25	6	0.97 (0.63,1.51)	**0.14**	2	2.22 (0.55,8.91)	0.16	4	2.78 (1.40,5.51)	0.30
Tooth level	2	0.47 (0.05,4.49)		2	**0.38 (0.18,0.78)***		3	0.95 (0.64,1.43)		2	0.46 (0.03,7.52)	
Incisors†	2	1.12 (0.59,2.14)	0.80	2	0.58 (0.04,7.71)	0.96	3	1.55 (0.94,2.58)	0.57	2	3.10 (1.21,7.93)	0.89
Molars	2	1.50 (0.32,7.05)		2	0.87 (0.15,5.02)		3	1.44 (0.38,5.38)		2	2.76 (0.74,10.36)	

* statistically significant Relative Risk for this subgroup (P < 0.05).

$ statistically significant difference between subgroups (P < 0.10).

† analyzing separately experimental arms from the same study that were initially pooled together for the main analysis.

A. a., Aggregatibacter actinomycetemcomitans; P. g., Porphyromonas gingivalis; P. i., Prevotella intermedia; T. f., Tanerrela forsythia; n, number of studies; RR, relative risk; CI, confidence interval; P_{SG}, P for differences among subgroups; FU, follow-up; mos, months.

*Appendix S9b***.** Subgroup analyses and meta-regressions from uncontrolled studies of the effect of orthodontic appliance removal on the subgingival microbiota of orthodontically treated patients.

	Prevalence of A. a.				**Prevalence of P. g.**				**Prevalence of P. i.**				**Prevalence of T. f.**		
Subgroup	**n**	**RR (95% CI)**	**P_{SG}**		**n**	**RR (95% CI)**	**P_{SG}**		**n**	**RR (95% CI)**	**P_{SG}**		**n**	**RR (95% CI)**	**P_{SG}**
Patient age	-	-	-		3	0.84 (0.07,10.81)	0.54		-	-	-		-	-	-
Male % of the sample	-	-	-		4	1.10 (0.65,1.88)	0.51		-	-	-		-	-	-
FU < 3 mos	-	-	-		3	0.87 (0.62,1.21)	**0.07**$^{\$}$		-	-	-		-	-	-
3 mos ≤ FU < 6 mos	-	-	-		2	0.49 (0.23,1.07)			-	-	-		-	-	-
FU ≥ 6 mos	-	-	-		1	**0.45 (0.26,0.77)***			-	-	-		-	-	-
Lingual brackets	-	-	-		-	-	-		-	-	-		-	-	-
Conventional brackets	-	-	-		-	-	-		-	-	-		-	-	-
Patient level†	-	-	-		2	0.67 (0.28,1.63)	0.55		-	-	-		-	-	-
Tooth level†	-	-	-		4	0.75 (0.53,1.04)			-	-	-		-	-	-

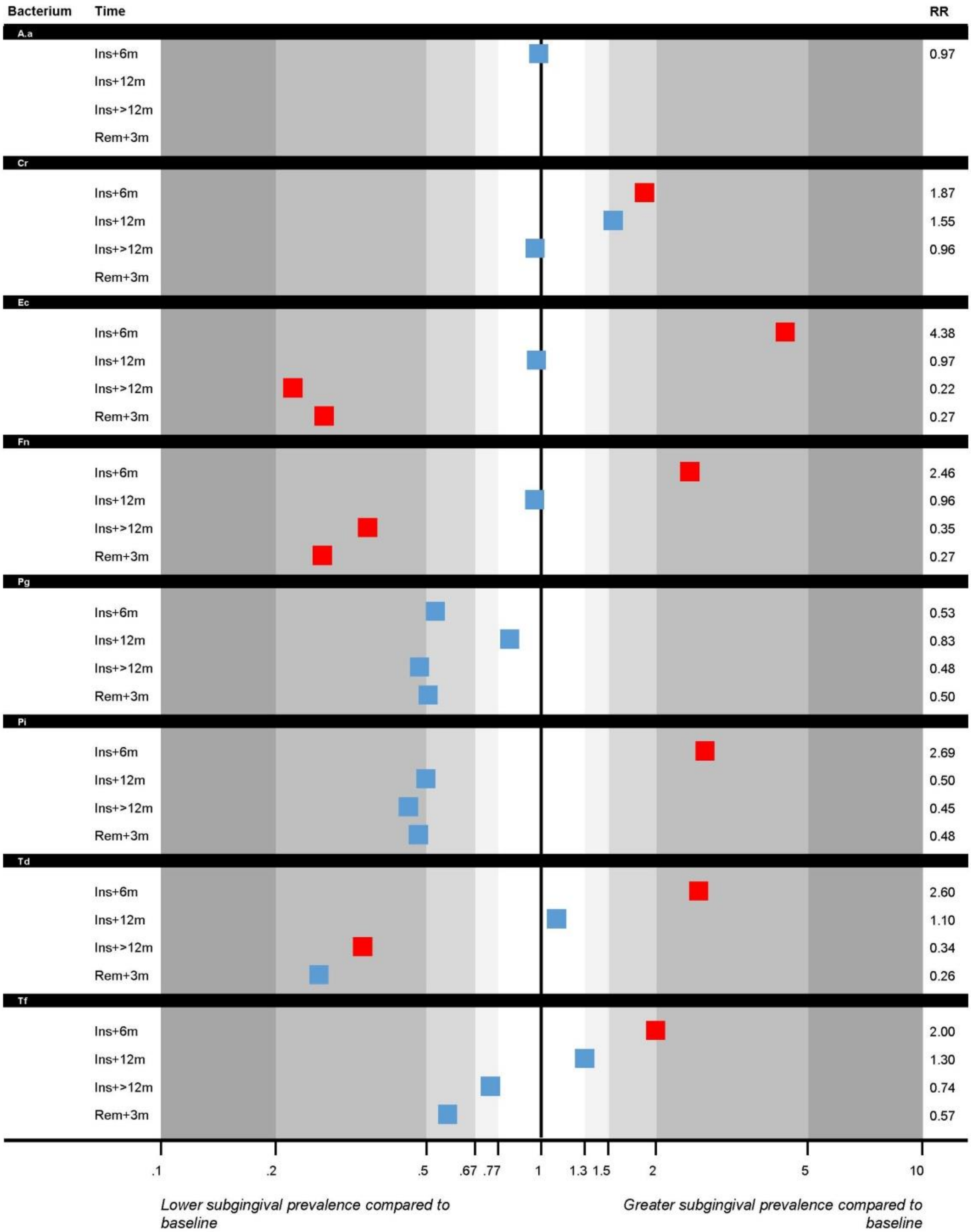
* statistically significant Relative Risk for this subgroup (P < 0.05).
$ statistically significant difference between subgroups (P < 0.10).
† analyzing separately experimental arms from the same study that were initially pooled together for the main analysis.
A. a., Aggregatibacter actinomycetemcomitans; CI, confidence interval; FU, follow-up; mos, months; n, number of studies; P. g., Porphyromonas gingivalis; P. i., Prevotella intermedia; P_{SG}, P for differences among subgroups; RR, relative risk; T. f., Tanerrela forsythia

Appendix S10. Sensitivity analysis by including the most precise half of eligible studies (i.e. those with the smallest standard error).

			Original analysis				**Sensitivity analysis**	
Comparison	**Outcome**	**Studies**	**RR (95% CI)**	**P**		**Studies**	**RR (95% CI)**	**P**
Appliance insertion (referent: before insertion)								
	Prevalence of *A.a.*	8	1.60 (0.58-4.41)	0.36		4	1.12 (0.81-1.53)	0.50
	Prevalence of *C.r.*	2	1.56 (1.24-1.95)	<0.001		NT		
	Prevalence of *E.c.*	3	0.99 (0.49-2.00)	0.98		NT		
	Prevalence of *F.n.*	2	1.02 (0.77-1.35)	0.90		NT		
	Prevalence of *P.g.*	8	0.82 (0.49,1.35)	0.43		4	0.82 (0.45-1.48)	0.50
	Prevalence of *P.i.*	5	1.23 (0.56-2.72)	0.60		3	1.53 (0.61-3.81)	0.36
	Prevalence of *T.d.*	2	1.41 (0.86-2.32)	0.17		NT		
	Prevalence of *T.f.*	6	1.83 (0.70,4.75)	0.22		3	2.17 (0.95-4.96)	0.07
Appliance removal (referent: before removal)								
	Prevalence of *A.a.*	4	0.33 (0.20-0.53)	<0.001		NT		
	Prevalence of *P.g.*	6	0.69 (0.51-0.94)	0.02		3	0.70 (0.43-1.14)	0.15
	Prevalence of *P.i.*	4	0.95 (0.61-1.49)	0.83		NT		
	Prevalence of *T.d.*	3	0.49 (0.36-0.65)	<0.001		NT		
	Prevalence of *T.f.*	4	0.68 (0.51-0.92)	0.01		NT		

A. a., Aggregatibacter actinomycetemcomitans; CI, confidence interval; P. g., Porphyromonas gingivalis; P. i., Prevotella intermedia; RR, relative risk; T. f., Tanerrela forsythia.

*Appendix S11***.** Results of the identified study Thornberg et al., 2009 that was excluded from the meta-analysis as it did not report overall detection of bacteria in the sulcus, but reported the presence of high bacterial counts in the sulcus. Results are presented as Relative Risks of each time point (from 6 to over 12 months after treatment and finally 6 months after appliance removal) compared to baseline (pre-insertion) status. Red boxed denote statistical significance at the 5% level.



*Appendix S12***.** Supplementary Information on the review

Author contributions

SNP,TE conceived the idea and wrote the first draft of the protocol. SNP, GMX, MTC, TE revised the protocol. SNP performed the literature searches, extracted search hits, and did screening by title. SNP and GMX did study selection by abstract and full-text, did data extraction, and assessed the risk of bias in duplicate, while TE resolved any conflicts that arose. SNP handled communications with trialists, performed the statistical analysis, and wrote the first draft of the manuscript. SNP, GMX, MTC, TE assisted in the interpretation of the results and revised the manuscript draft. SNP submitted the manuscript, is the guarantor and responsible for the accuracy of the data and for future updates of the review.

***Post hoc* changes to the protocol**

- A novel Paule Mandel estimator had been published after initial protocol writing. Since it has been reported to outperform the standard DerSimonian and Laird estimator for random-effects variance this was preferred for all analyses.
- Several subgroup and sensitivity analyses were planned in the PROSPERO protocol, but could not be performed due to limited data.
- A vast number of outcomes were finally identified afte study selection. In order to keep the risk of false positives due to multiple testing low, we limited our analyses to only a choice of outcome that were mostly reported by included studies. However, all outcomes that were found have been transparently listed in the Appendix.